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(54) **METHODS OF TREATING UROGENITAL-NEUROLOGICAL DISORDERS USING MODIFIED CLOSTRIDIAL TOXINS**

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(52) **U.S. Cl.**
CPC ***A61K 38/16*** (2013.01)

(58) **Field of Classification Search**
None
See application file for complete search history.

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(57) **ABSTRACT**

The present specification discloses modified Clostridial toxins, compositions comprising such toxins and methods of treating urogenital-neurological disorders in a mammal using such modified Clostridial toxins and compositions.

11 Claims, 7 Drawing Sheets

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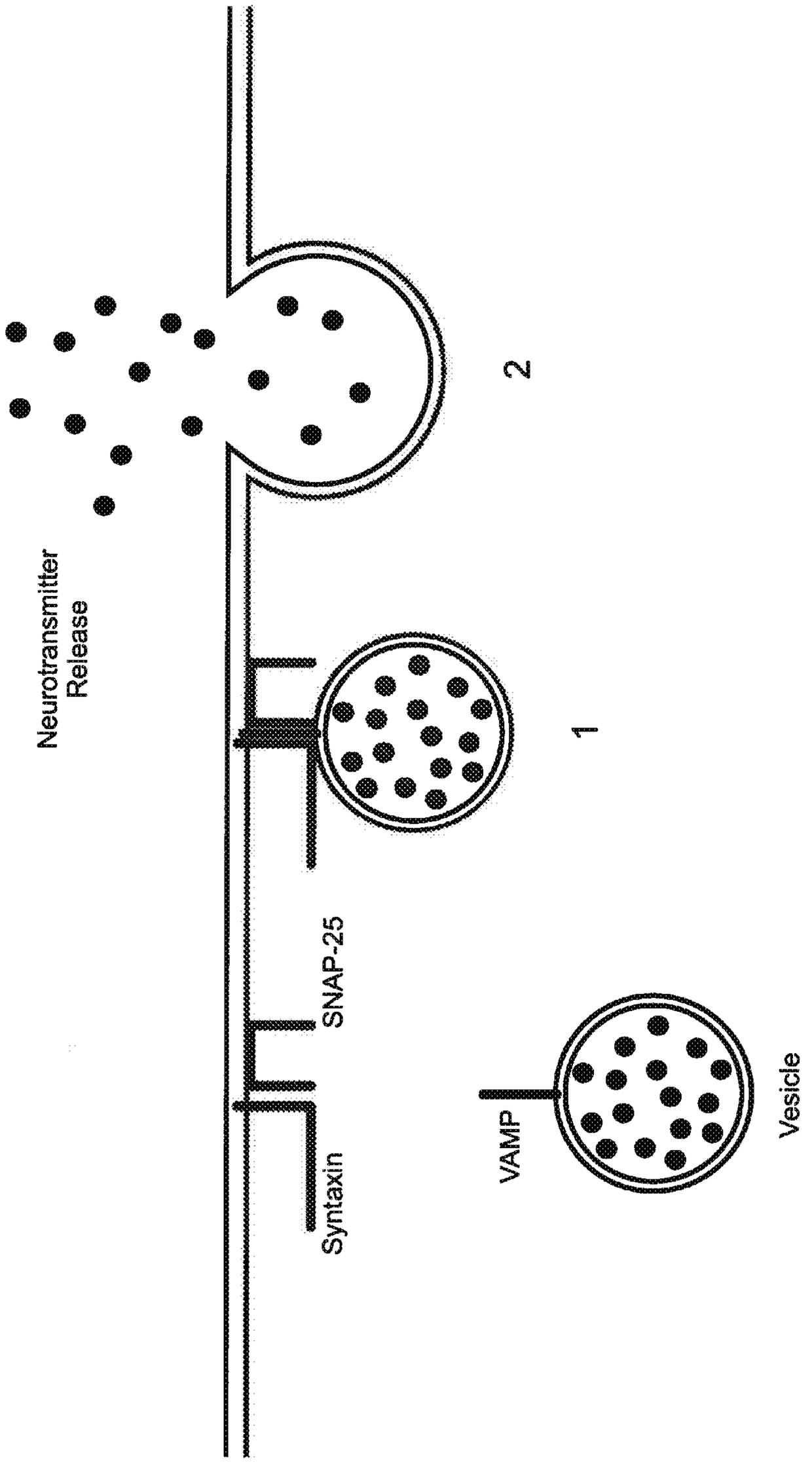

Neurotransmitter Release
2
1
SNAP-25
Syntaxin
VAMP
Vesicle

FIG. 1A.

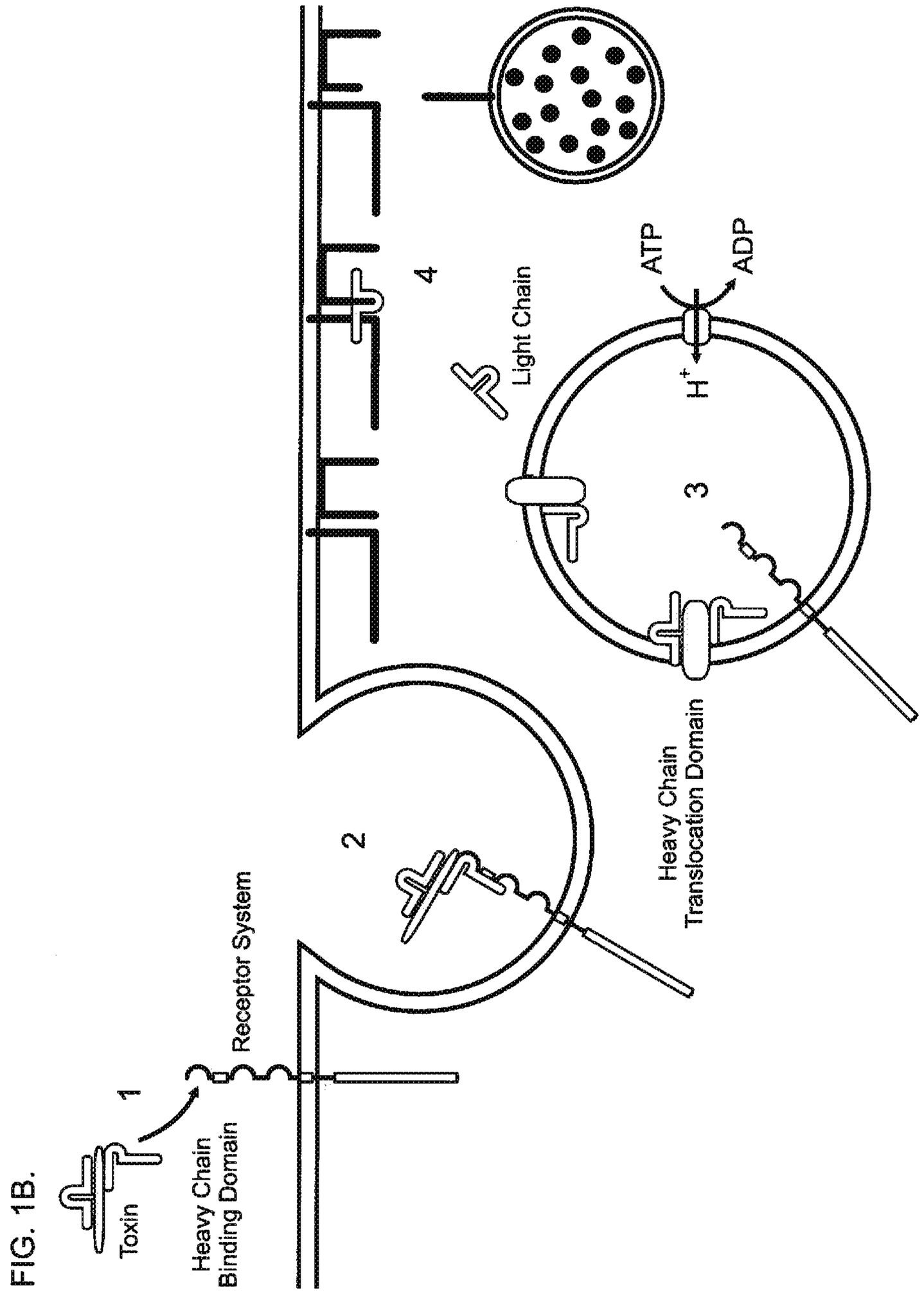

Receptor System
Toxin
1
Heavy Chain
Binding Domain
2
Heavy Chain
Translocation Domain
3
Light Chain
4
ATP
ADP
H+

FIG. 1B.

FIG. 2.

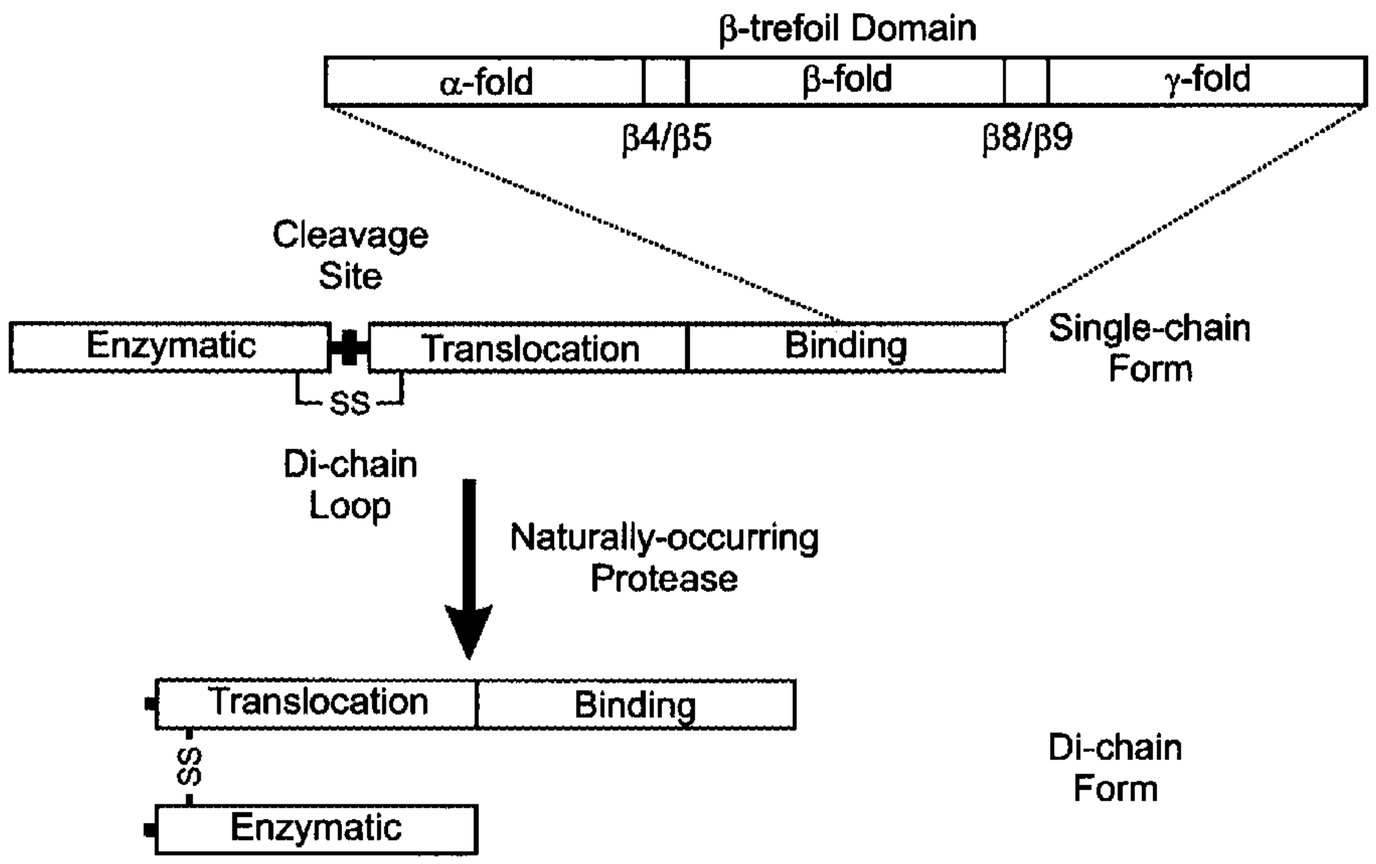

β-trefoil Domain
α-fold
β-fold
γ-fold
β4/β5
β8/β9
Cleavage Site
Enzymatic
Translocation
Binding
Single-chain Form
SS
Di-chain Loop
Naturally-occurring Protease
Translocation
Binding
SS
Enzymatic
Di-chain Form

FIG. 3A.

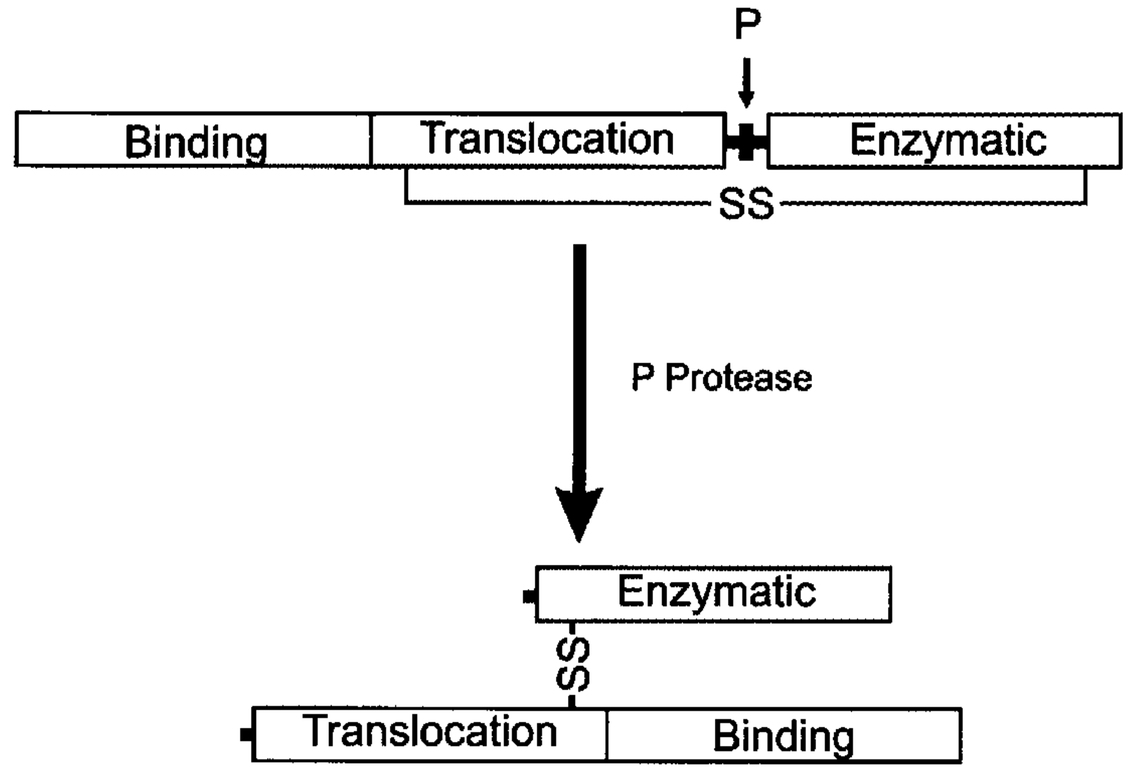

P
Binding
Translocation
Enzymatic
SS
P Protease
Enzymatic
SS
Translocation
Binding

FIG. 3B.

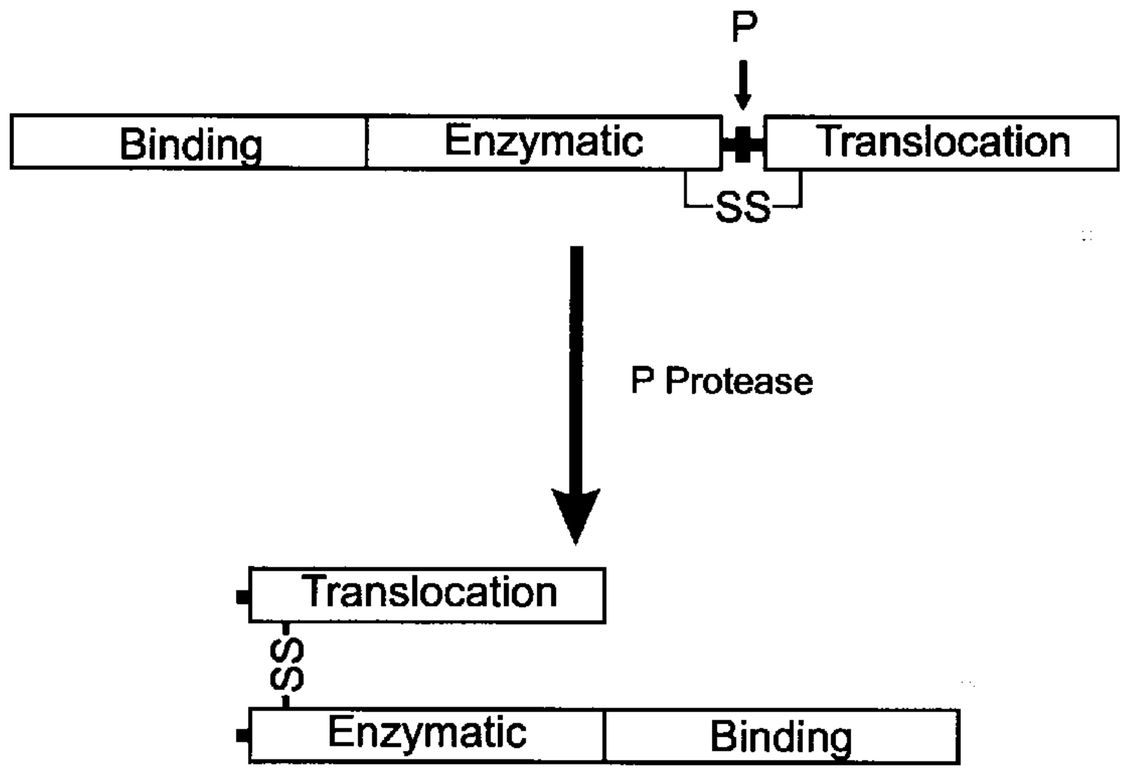

P
Binding
Enzymatic
Translocation
SS
P Protease
Translocation
SS
Enzymatic
Binding

FIG. 4A.

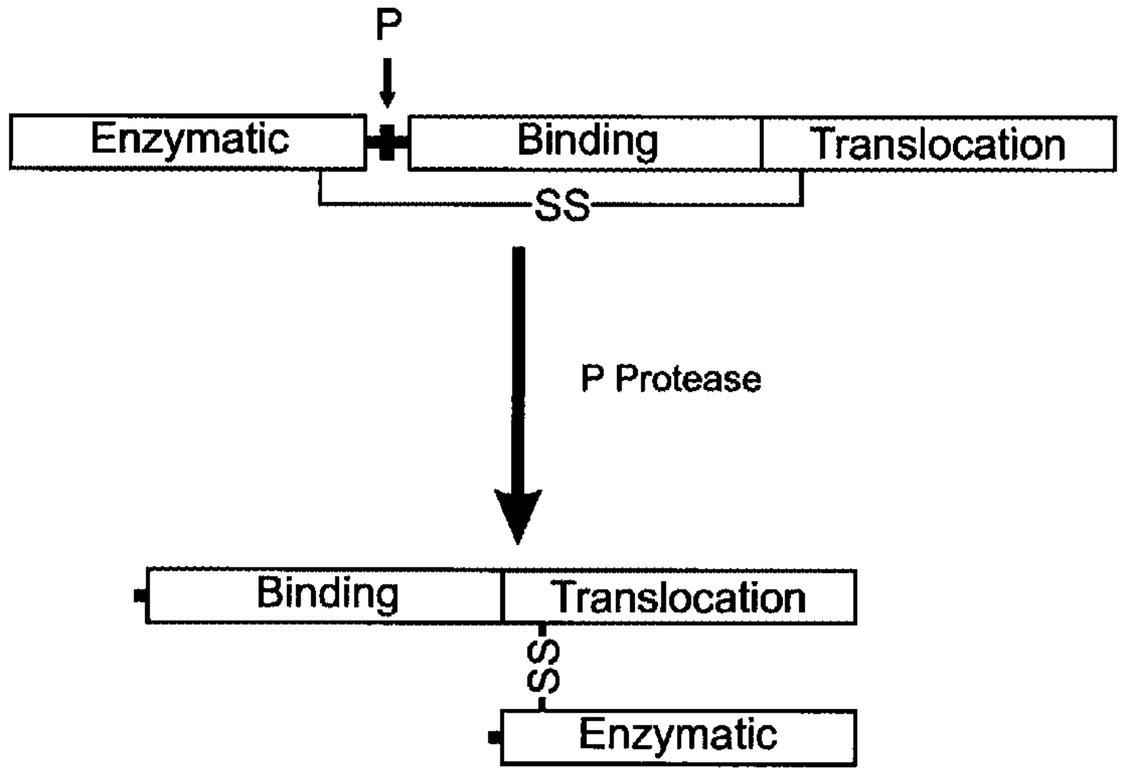
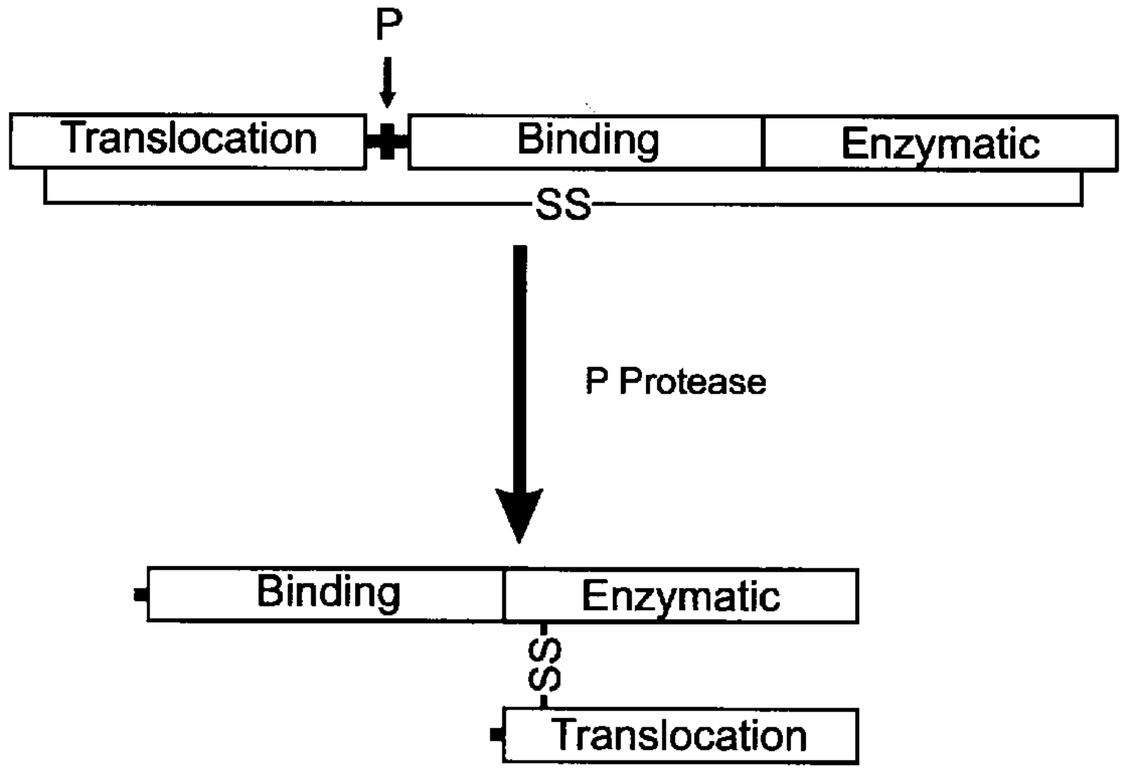

P
Enzymatic
Binding
Translocation
SS
P Protease
Binding
Translocation
SS
Enzymatic

FIG. 4B.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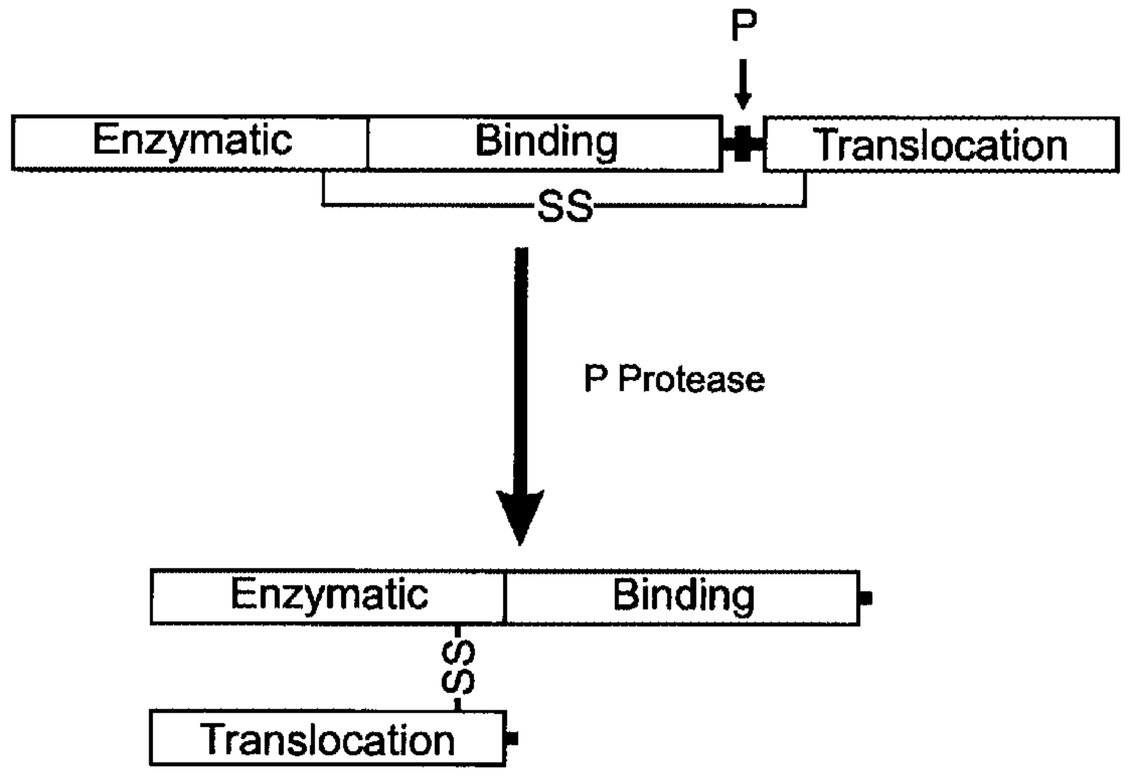
Translocation
Binding
Enzymatic
SS
P Protease
Binding
Enzymatic
SS
Translocation

FIG. 4C.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Enzymatic
Binding
Translocation
SS
P Protease
Enzymatic
Binding
SS
Translocation

FIG. 4D.

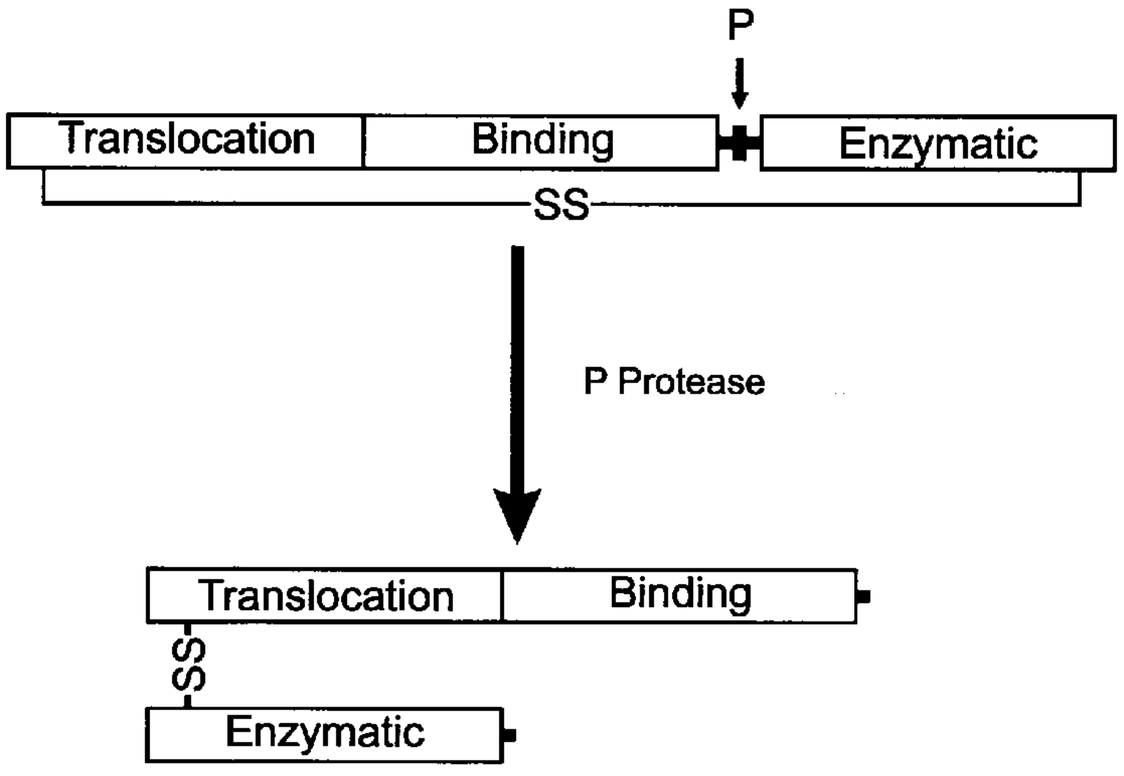

P
Translocation
Binding
Enzymatic
SS
P Protease
Translocation
Binding
SS
Enzymatic

FIG. 5A.

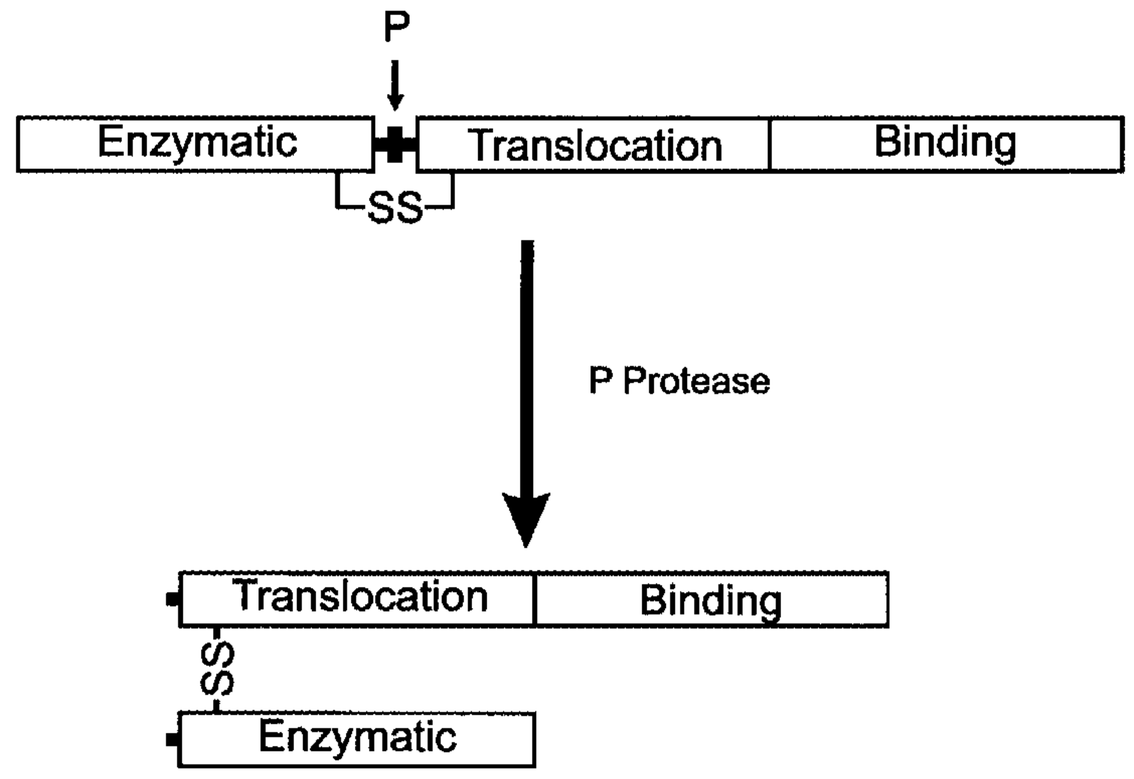
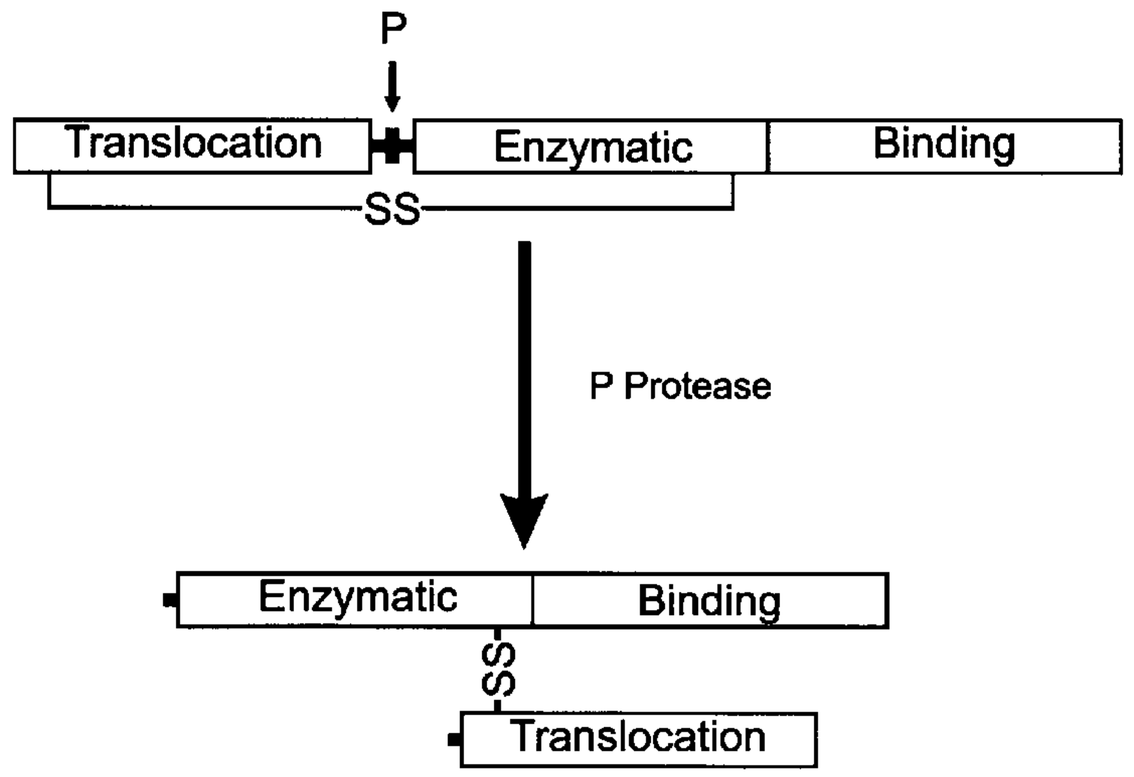

P
Enzymatic
Translocation
Binding
SS
P Protease
Translocation
Binding
SS
Enzymatic

FIG. 5B.


P
Translocation
Enzymatic
Binding
SS
P Protease
Enzymatic
Binding
SS
Translocation

METHODS OF TREATING UROGENITAL-NEUROLOGICAL DISORDERS USING MODIFIED CLOSTRIDIAL TOXINS

This patent application claims priority pursuant to 35 U.S.C. §119(e) to U.S. Provisional Patent Application Ser. No. 60/982,021 filed Oct. 23, 2007, and U.S. Provisional Patent Application Ser. No. 61/076,228 filed Jun. 27, 2008, each of which is hereby incorporated by reference in its entirety.

The ability of Clostridial toxins, such as, e.g., Botulinum neurotoxins (BoNTs), BoNT/A, BoNT/B, BoNT/C1, BoNT/D, BoNT/E, BoNT/F and BoNT/G, and Tetanus neurotoxin (TeNT), to inhibit neuronal transmission are being exploited in a wide variety of therapeutic and cosmetic applications, see e.g., William J. Lipham, COSMETIC AND CLINICAL APPLICATIONS OF BOTULINUM TOXIN (Slack, Inc., 2004). Clostridial toxins commercially available as pharmaceutical compositions include, BoNT/A preparations, such as, e.g., BOTOX® (Allergan, Inc., Irvine, Calif.), Dysport®/Reloxin®, (Beaufour Ipsen, Porton Down, England), Linurase® (Prollenium, Inc., Ontario, Canada), Neuronox® (Medy-Tox, Inc., Ochang-myeon, South Korea) BTX-A (Lanzhou Institute Biological Products, China) and Xeomin® (Merz Pharmaceuticals, GmbH., Frankfurt, Germany); and BoNT/B preparations, such as, e.g., MyoBloc™/NeuroBloc™ (Elan Pharmaceuticals, San Francisco, Calif.). As an example, BOTOX® is currently approved in one or more countries for the following indications: achalasia, adult spasticity, anal fissure, back pain, blepharospasm, bruxism, cervical dystonia, essential tremor, glabellar lines or hyperkinetic facial lines, headache, hemifacial spasm, hyperactivity of bladder, hyperhidrosis, juvenile cerebral palsy, multiple sclerosis, myoclonic disorders, nasal labial lines, spasmodic dysphonia, strabismus and VII nerve disorder.

Clostridial toxin therapies are successfully used for many indications. Generally, administration of a Clostridial toxin treatment is well tolerated. However, toxin administration in some applications can be challenging because of the larger doses required to achieve a beneficial effect. Larger doses can increase the likelihood that the toxin may move through the interstitial fluids and the circulatory systems, such as, e.g., the cardiovascular system and the lymphatic system, of the body, resulting in the undesirable dispersal of the toxin to areas not targeted for toxin treatment. Such dispersal can lead to undesirable side effects, such as, e.g., inhibition of neurotransmitter release in neurons not targeted for treatment or paralysis of a muscle not targeted for treatment. For example, a patient administered a therapeutically effective amount of a BoNT/A treatment into the neck muscles for torticollis may develop dysphagia because of dispersal of the toxin into the oropharynx. As another example, a patient administered a therapeutically effective amount of a BoNT/A treatment into the bladder for overactive bladder may develop dry month and/or dry eyes. Thus, there remains a need for improved Clostridial toxins that are effective at the site of treatment, but have negligible to minimal effects in areas not targeted for a toxin treatment.

A Clostridial toxin treatment inhibits neurotransmitter release by disrupting the exocytotic process used to secret the neurotransmitter into the synaptic cleft. There is a great desire by the pharmaceutical industry to expand the use of Clostridial toxin therapies beyond its current myo-relaxant applications to treat sensory nerve-based ailment, such as, e.g., various kinds of chronic pain, neurogenic inflammation and urogenital disorders, as well as other disorders, such as,

e.g., pancreatitis. One approach that is currently being exploited to expand Clostridial toxin-based therapies involves modifying a Clostridial toxin so that the modified toxin has an altered cell targeting capability for a non-Clostridial toxin target cell. This re-targeted capability is achieved by replacing a naturally-occurring targeting domain of a Clostridial toxin with a targeting domain showing a selective binding activity for a receptor present on a non-Clostridial toxin target cell. Such modifications to a targeting domain result in a modified toxin that is able to selectively bind to a non-Clostridial toxin receptor (target receptor) present on a non-Clostridial toxin target cell (re-targeted). A modified Clostridial toxin with a targeting activity for a non-Clostridial toxin target cell can bind to a receptor present on the non-Clostridial toxin target cell, translocate into the cytoplasm, and exert its proteolytic effect on the SNARE complex of the non-Clostridial toxin target cell.

The present specification discloses modified Clostridial toxin compositions and methods for treating an individual suffering from a nociceptive sensory neuron-mediated urogenital disorder. This is accomplished by administering a therapeutically effective amount of a composition comprising a modified Clostridial toxin to an individual in need thereof. The disclosed methods provide a safe, inexpensive, out patient-based treatment for the treatment of urogenital-neurological disorders.

Thus, aspects of the present invention provide a composition comprising a modified Clostridial toxin comprising an opioid peptide binding domain, a Clostridial toxin translocation domain and a Clostridial toxin enzymatic domain. Modified Clostridial toxins useful for the development of such compositions are described in, e.g., Steward, L. E. et al., Modified Clostridial Toxins with Enhanced Translocation Capabilities and Altered Targeting Activity For Non-Clostridial Toxin Target Cells, U.S. patent application Ser. No. 11/776,075 (Jul. 11, 2007); Dolly, J. O. et al., Activatable Clostridial Toxins, U.S. patent application Ser. No. 11/829,475 (Jul. 27, 2007); Foster, K. A. et al., Fusion Proteins, International Patent Publication WO 2006/059093 (Jun. 8, 2006); and Foster, K. A. et al., Non-Cytotoxic Protein Conjugates, International Patent Publication WO 2006/059105 (Jun. 8, 2006), each of which is incorporated by reference in its entirety. A composition comprising a modified Clostridial toxin can be a pharmaceutical composition. Such a pharmaceutical composition can comprise, in addition to a modified Clostridial toxin, a pharmaceutical carrier, a pharmaceutical component, or both.

Other aspects of the present invention provide a method of treating urogenital-neurological disorder in a mammal, the method comprising the step of administering to the mammal a therapeutically effective amount of a composition including a modified Clostridial toxin comprising an opioid peptide binding domain, a Clostridial toxin translocation domain and a Clostridial toxin enzymatic domain. It is envisioned that any modified Clostridial toxin disclosed in the present specification can be used, including those disclosed in, e.g., Steward, supra, (2007); Dolly, supra, (2007); Foster, supra, WO 2006/059093 (2006); and Foster, supra, WO 2006/059105 (Jun. 8, 2006).

Other aspects of the present invention provide a use of a modified Clostridial toxin comprising an opioid peptide binding domain, a Clostridial toxin translocation domain and a Clostridial toxin enzymatic domain in the manufacturing a medicament for treating urogenital-neurological disorder in a mammal, the use comprising the step of administering to the mammal a therapeutically effective amount of a composition including a modified Clostridial toxin comprising an opioid

peptide binding domain, a Clostridial toxin translocation domain and a Clostridial toxin enzymatic domain. It is envisioned that any modified Clostridial toxin disclosed in the present specification can be used, including those disclosed in, e.g., Steward, supra, (2007); Dolly, supra, (2007); Foster, supra, WO 2006/059093 (2006); and Foster, supra, WO 2006/059105 (Jun. 8, 2006).

Other aspects of the present invention provide a modified Clostridial toxin comprising an opioid peptide binding domain, a Clostridial toxin translocation domain and a Clostridial toxin enzymatic domain for treating urogenital-neurological disorder in a mammal, the use comprising the step of administering to the mammal a therapeutically effective amount of a composition including a modified Clostridial toxin comprising an opioid peptide binding domain, a Clostridial toxin translocation domain and a Clostridial toxin enzymatic domain. It is envisioned that any modified Clostridial toxin disclosed in the present specification can be used, including those disclosed in, e.g., Steward, supra, (2007); Dolly, supra, (2007); Foster, supra, WO 2006/059093 (2006); and Foster, supra, WO 2006/059105 (Jun. 8, 2006).

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** shows a schematic of the current paradigm of neurotransmitter release and Clostridial toxin intoxication in a central and peripheral neuron. FIG. **1**A shows a schematic for the neurotransmitter release mechanism of a central and peripheral neuron. The release process can be described as comprising two steps: 1) vesicle docking, where the vesicle-bound SNARE protein of a vesicle containing neurotransmitter molecules associates with the membrane-bound SNARE proteins located at the plasma membrane; and 2) neurotransmitter release, where the vesicle fuses with the plasma membrane and the neurotransmitter molecules are exocytosed. FIG. **1**B shows a schematic of the intoxication mechanism for tetanus and botulinum toxin activity in a central and peripheral neuron. This intoxication process can be described as comprising four steps: 1) receptor binding, where a Clostridial toxin binds to a Clostridial receptor system and initiates the intoxication process; 2) complex internalization, where after toxin binding, a vesicle containing the toxin/receptor system complex is endocytosed into the cell; 3) light chain translocation, where multiple events are thought to occur, including, e.g., changes in the internal pH of the vesicle, formation of a channel pore comprising the HN domain of the Clostridial toxin heavy chain, separation of the Clostridial toxin light chain from the heavy chain, and release of the active light chain and 4) enzymatic target modification, where the activate light chain of Clostridial toxin proteolytically cleaves its target SNARE substrate, such as, e.g., SNAP-25, VAMP or Syntaxin, thereby preventing vesicle docking and neurotransmitter release.

FIG. **2** shows the domain organization of naturally-occurring Clostridial toxins. The single-chain form depicts the amino to carboxyl linear organization comprising an enzymatic domain, a translocation domain, and an opioid peptide binding domain. The di-chain loop region located between the translocation and enzymatic domains is depicted by the double SS bracket. This region comprises an endogenous di-chain loop protease cleavage site that upon proteolytic cleavage with a naturally-occurring protease, such as, e.g., an endogenous Clostridial toxin protease or a naturally-occurring protease produced in the environment, converts the single-chain form of the toxin into the di-chain form. Above the single-chain form, the HCC region of the Clostridial toxin binding domain is depicted. This region comprises the β-trefoil domain which comprises in a amino to carboxyl linear organization an α-fold, a β4/β5 hairpin turn, a β-fold, a β8/β9 hairpin turn and a γ-fold.

FIG. **3** shows modified Clostridial toxins with an enhanced targeting domain located at the amino terminus of the modified toxin. FIG. **3**A depicts the single-chain polypeptide form of a modified Clostridial toxin with an amino to carboxyl linear organization comprising a binding element, a translocation element, a di-chain loop region comprising an exogenous protease cleavage site (P), and a therapeutic element. Upon proteolytic cleavage with a P protease, the single-chain form of the toxin is converted to the di-chain form. FIG. **3**B depicts the single polypeptide form of a modified Clostridial toxin with an amino to carboxyl linear organization comprising a binding element, a therapeutic element, a di-chain loop region comprising an exogenous protease cleavage site (P), and a translocation element. Upon proteolytic cleavage with a P protease, the single-chain form of the toxin is converted to the di-chain form.

FIG. **4** shows modified Clostridial toxins with an enhanced targeting domain located between the other two domains. FIG. **4**A depicts the single polypeptide form of a modified Clostridial toxin with an amino to carboxyl linear organization comprising a therapeutic element, a di-chain loop region comprising an exogenous protease cleavage site (P), a binding element, and a translocation element. Upon proteolytic cleavage with a P protease, the single-chain form of the toxin is converted to the di-chain form. FIG. **4**B depicts the single polypeptide form of a modified Clostridial toxin with an amino to carboxyl linear organization comprising a translocation element, a di-chain loop region comprising an exogenous protease cleavage site (P), a binding element, and a therapeutic element. Upon proteolytic cleavage with a P protease, the single-chain form of the toxin is converted to the di-chain form. FIG. **4**C depicts the single polypeptide form of a modified Clostridial toxin with an amino to carboxyl linear organization comprising a therapeutic element, a binding element, a di-chain loop region comprising an exogenous protease cleavage site (P), and a translocation element. Upon proteolytic cleavage with a P protease, the single-chain form of the toxin is converted to the di-chain form. FIG. **4**D depicts the single polypeptide form of a modified Clostridial toxin with an amino to carboxyl linear organization comprising a translocation element, a binding element, a di-chain loop region comprising an exogenous protease cleavage site (P), and a therapeutic element. Upon proteolytic cleavage with a P protease, the single-chain form of the toxin is converted to the di-chain form.

FIG. **5** shows modified Clostridial toxins with an enhanced targeting domain located at the carboxyl terminus of the modified toxin. FIG. **5**A depicts the single polypeptide form of a modified Clostridial toxin with an amino to carboxyl linear organization comprising a therapeutic element, a di-chain loop region comprising an exogenous protease cleavage site (P), a translocation element, and a binding element. Upon proteolytic cleavage with a P protease, the single-chain form of the toxin is converted to the di-chain form. FIG. **5**B depicts the single polypeptide form of a modified Clostridial toxin with an amino to carboxyl linear organization comprising a translocation element, a di-chain loop region comprising an exogenous protease cleavage site (P), a therapeutic element, and a binding element. Upon proteolytic cleavage with a P protease, the single-chain form of the toxin is converted to the di-chain form.

Aspects of the present invention provide, in part, a modified Clostridial toxin. As used herein, a "modified Clostridial toxin" means any molecule comprising an opioid peptide

binding domain, a Clostridial toxin translocation domain and a Clostridial toxin enzymatic domain. Exemplary modified Clostridial toxins useful to practice aspects of the present invention are disclosed in, e.g., Steward, supra, (2007); Dolly, supra, (2007); Foster, supra, WO 2006/059093 (2006); Foster, supra, WO 2006/059105 (Jun. 8, 2006).

Clostridia toxins produced by *Clostridium botulinum, Clostridium tetani, Clostridium baratii* and *Clostridium butyricum* are the most widely used in therapeutic and cosmetic treatments of humans and other mammals. Strains of *C. botulinum* produce seven antigenically-distinct types of Botulinum toxins (BoNTs), which have been identified by investigating botulism outbreaks in man (BoNT/A, /B, /E and /F), animals (BoNT/C1 and /D), or isolated from soil (BoNT/G). BoNTs possess approximately 35% amino acid identity with each other and share the same functional domain organization and overall structural architecture. It is recognized by those of skill in the art that within each type of Clostridial toxin there can be subtypes that differ somewhat in their amino acid sequence, and also in the nucleic acids encoding these proteins. For example, there are presently four BoNT/A subtypes, BoNT/A1, BoNT/A2, BoNT/A3 and BoNT/A4, with specific subtypes showing approximately 89% amino acid identity when compared to another BoNT/A subtype. While all seven BoNT serotypes have similar structure and pharmacological properties, each also displays heterogeneous bacteriological characteristics. In contrast, tetanus toxin (TeNT) is produced by a uniform group of *C. tetani*. Two other species of *Clostridia, C. baratii* and *C. butyricum*, also produce toxins, BaNT and BuNT respectively, which are similar to BoNT/F and BoNT/E, respectively.

Each mature di-chain molecule comprises three functionally distinct domains: 1) an enzymatic domain located in the LC that includes a metalloprotease region containing a zinc-dependent endopeptidase activity which specifically targets core components of the neurotransmitter release apparatus; 2) a translocation domain contained within the amino-terminal half of the HC (H_N) that facilitates release of the LC from intracellular vesicles into the cytoplasm of the target cell; and 3) a binding domain found within the carboxyl-terminal half of the HC (H_C) that determines the binding activity and binding specificity of the toxin to the receptor complex located at the surface of the target cell. The H_C domain comprises two distinct structural features of roughly equal size that indicate function and are designated the H_{CN} and H_{CC} subdomains. Table 1 gives approximate boundary regions for each domain found in exemplary Clostridial toxins.

TABLE 1

Clostridial Toxin Reference Sequences and Regions

Toxin	SEQ ID NO:	LC	H_N	H_C
BoNT/A	1	M1-K448	A449-K871	N872-L1296
BoNT/B	2	M1-K441	A442-S858	E859-E1291
BoNT/C1	3	M1-K449	T450-N866	N867-E1291
BoNT/D	4	M1-R445	D446-N862	S863-E1276
BoNT/E	5	M1-R422	K423-K845	R846-K1252
BoNT/F	6	M1-K439	A440-K864	K865-E1274
BoNT/G	7	M1-K446	S447-S863	N864-E1297
TeNT	8	M1-A457	S458-V879	I880-D1315
BaNT	9	M1-K431	N432-I857	I858-E1268
BuNT	10	M1-R422	K423-I847	Y1086-K1251

The binding, translocation and enzymatic activity of these three functional domains are all necessary for toxicity. While all details of this process are not yet precisely known, the overall cellular intoxication mechanism whereby Clostridial toxins enter a neuron and inhibit neurotransmitter release is similar, regardless of serotype or subtype. Although the applicants have no wish to be limited by the following description, the intoxication mechanism can be described as comprising at least four steps: 1) receptor binding, 2) complex internalization, 3) light chain translocation, and 4) enzymatic target modification (see FIG. **1**). The process is initiated when the H_C domain of a Clostridial toxin binds to a toxin-specific receptor system located on the plasma membrane surface of a target cell. The binding specificity of a receptor complex is thought to be achieved, in part, by specific combinations of gangliosides and protein receptors that appear to distinctly comprise each Clostridial toxin receptor complex. Once bound, the toxin/receptor complexes are internalized by endocytosis and the internalized vesicles are sorted to specific intracellular routes. The translocation step appears to be triggered by the acidification of the vesicle compartment. This process seems to initiate two important pH-dependent structural rearrangements that increase hydrophobicity and promote formation di-chain form of the toxin. Once activated, light chain endopeptidase of the toxin is released from the intracellular vesicle into the cytosol where it appears to specifically targets one of three known core components of the neurotransmitter release apparatus. These core proteins, vesicle-associated membrane protein (VAMP)/synaptobrevin, synaptosomal-associated protein of 25 kDa (SNAP-25) and Syntaxin, are necessary for synaptic vesicle docking and fusion at the nerve terminal and constitute members of the soluble N-ethylmaleimide-sensitive factor-attachment protein-receptor (SNARE) family. BoNT/A and BoNT/E cleave SNAP-25 in the carboxyl-terminal region, releasing a nine or twenty-six amino acid segment, respectively, and BoNT/C1 also cleaves SNAP-25 near the carboxyl-terminus. The botulinum serotypes BoNT/B, BoNT/D, BoNT/F and BoNT/G, and tetanus toxin, act on the conserved central portion of VAMP, and release the amino-terminal portion of VAMP into the cytosol. BoNT/C1 cleaves syntaxin at a single site near the cytosolic membrane surface. The selective proteolysis of synaptic SNAREs accounts for the block of neurotransmitter release caused by Clostridial toxins in vivo. The SNARE protein targets of Clostridial toxins are common to exocytosis in a variety of non-neuronal types; in these cells, as in neurons, light chain peptidase activity inhibits exocytosis, see, e.g., Yann Humeau et al., *How Botulinum and Tetanus Neurotoxins Block Neurotransmitter Release,* 82(5) Biochimie. 427-446 (2000); Kathryn Turton et al., *Botulinum and Tetanus Neurotoxins: Structure, Function and Therapeutic Utility,* 27(11) Trends Biochem. Sci. 552-558. (2002); Giovanna Lalli et al., *The Journey of Tetanus and Botulinum Neurotoxins in Neurons,* 11(9) Trends Microbiol. 431-437, (2003).

In an aspect of the invention, a modified Clostridial toxin comprises, in part, a Clostridial toxin enzymatic domain. As used herein, the term "Clostridial toxin enzymatic domain" means any Clostridial toxin polypeptide that can execute the enzymatic target modification step of the intoxication process. Thus, a Clostridial toxin enzymatic domain specifically targets a Clostridial toxin substrate and encompasses the proteolytic cleavage of a Clostridial toxin substrate, such as, e.g., SNARE proteins like a SNAP-25 substrate, a VAMP substrate and a Syntaxin substrate. Non-limiting examples of a Clostridial toxin enzymatic domain include, e.g., a BoNT/A enzymatic domain, a BoNT/B enzymatic domain, a BoNT/C1 enzymatic domain, a BoNT/D enzymatic domain, a BoNT/E enzymatic domain, a BoNT/F enzymatic domain, a BoNT/G enzymatic domain, a TeNT enzymatic domain, a BaNT enzymatic domain, and a BuNT enzymatic domain.

Other non-limiting examples of a Clostridial toxin enzymatic domain include, e.g., amino acids 1-448 of SEQ ID NO: 1, amino acids 1-441 of SEQ ID NO: 2, amino acids 1-449 of SEQ ID NO: 3, amino acids 1-445 of SEQ ID NO: 4, amino acids 1-422 of SEQ ID NO: 5, amino acids 1-439 of SEQ ID NO: 6, amino acids 1-446 of SEQ ID NO: 7, amino acids 1-457 of SEQ ID NO: 8, amino acids 1-431 of SEQ ID NO: 9, and amino acids 1-422 of SEQ ID NO: 10.

A Clostridial toxin enzymatic domain includes, without limitation, naturally occurring Clostridial toxin enzymatic domain variants, such as, e.g., Clostridial toxin enzymatic domain isoforms and Clostridial toxin enzymatic domain subtypes; non-naturally occurring Clostridial toxin enzymatic domain variants, such as, e.g., conservative Clostridial toxin enzymatic domain variants, non-conservative Clostridial toxin enzymatic domain variants, Clostridial toxin enzymatic domain chimerics, active Clostridial toxin enzymatic domain fragments thereof, or any combination thereof.

As used herein, the term "Clostridial toxin enzymatic domain variant," whether naturally-occurring or non-naturally-occurring, means a Clostridial toxin enzymatic domain that has at least one amino acid change from the corresponding region of the disclosed reference sequences (Table 1) and can be described in percent identity to the corresponding region of that reference sequence. Unless expressly indicated, Clostridial toxin enzymatic domain variants useful to practice disclosed embodiments are variants that execute the enzymatic target modification step of the intoxication process. As non-limiting examples, a BoNT/A enzymatic domain variant comprising amino acids 1-448 of SEQ ID NO: 1 will have at least one amino acid difference, such as, e.g., an amino acid substitution, deletion or addition, as compared to the amino acid region 1-448 of SEQ ID NO: 1; a BoNT/B enzymatic domain variant comprising amino acids 1-441 of SEQ ID NO: 2 will have at least one amino acid difference, such as, e.g., an amino acid substitution, deletion or addition, as compared to the amino acid region 1-441 of SEQ ID NO: 2; a BoNT/C1 enzymatic domain variant comprising amino acids 1-449 of SEQ ID NO: 3 will have at least one amino acid difference, such as, e.g., an amino acid substitution, deletion or addition, as compared to the amino acid region 1-449 of SEQ ID NO: 3; a BoNT/D enzymatic domain variant comprising amino acids 1-445 of SEQ ID NO: 4 will have at least one amino acid difference, such as, e.g., an amino acid substitution, deletion or addition, as compared to the amino acid region 1-445 of SEQ ID NO: 4; a BoNT/E enzymatic domain variant comprising amino acids 1-422 of SEQ ID NO: 5 will have at least one amino acid difference, such as, e.g., an amino acid substitution, deletion or addition, as compared to the amino acid region 1-422 of SEQ ID NO: 5; a BoNT/F enzymatic domain variant comprising amino acids 1-439 of SEQ ID NO: 6 will have at least one amino acid difference, such as, e.g., an amino acid substitution, deletion or addition, as compared to the amino acid region 1-439 of SEQ ID NO: 6; a BoNT/G enzymatic domain variant comprising amino acids 1-446 of SEQ ID NO: 7 will have at least one amino acid difference, such as, e.g., an amino acid substitution, deletion or addition, as compared to the amino acid region 1-446 of SEQ ID NO: 7; and a TeNT enzymatic domain variant comprising amino acids 1-457 of SEQ ID NO: 8 will have at least one amino acid difference, such as, e.g., an amino acid substitution, deletion or addition, as compared to the amino acid region 1-457 of SEQ ID NO: 8.

It is recognized by those of skill in the art that within each serotype of Clostridial toxin there can be naturally occurring Clostridial toxin enzymatic domain variants that differ somewhat in their amino acid sequence, and also in the nucleic acids encoding these proteins. For example, there are presently five BoNT/A subtypes, BoNT/A1, BoNT/A2, BoNT/A3, BoNT/A4, and BoNT/A5, with specific enzymatic domain subtypes showing approximately 95% amino acid identity when compared to another BoNT/A enzymatic domain subtype. As used herein, the term "naturally occurring Clostridial toxin enzymatic domain variant" means any Clostridial toxin enzymatic domain produced by a naturally-occurring process, including, without limitation, Clostridial toxin enzymatic domain isoforms produced from alternatively-spliced transcripts, Clostridial toxin enzymatic domain isoforms produced by spontaneous mutation and Clostridial toxin enzymatic domain subtypes. A naturally occurring Clostridial toxin enzymatic domain variant can function in substantially the same manner as the reference Clostridial toxin enzymatic domain on which the naturally occurring Clostridial toxin enzymatic domain variant is based, and can be substituted for the reference Clostridial toxin enzymatic domain in any aspect of the present invention. A naturally occurring Clostridial toxin enzymatic domain variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, ten or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids, 50 or more amino acids or 100 or more amino acids from the reference Clostridial toxin enzymatic domain on which the naturally occurring Clostridial toxin enzymatic domain variant is based. A naturally occurring Clostridial toxin enzymatic domain variant can also substitute at least 10 contiguous amino acids, at least 15 contiguous amino acids, at least 20 contiguous amino acids, or at least 25 contiguous amino acids from the reference Clostridial toxin enzymatic domain on which the naturally occurring Clostridial toxin enzymatic domain variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference Clostridial toxin enzymatic domain on which the naturally occurring Clostridial toxin enzymatic domain variant is based.

A non-limiting examples of a naturally occurring Clostridial toxin enzymatic domain variant is a Clostridial toxin enzymatic domain isoform such as, e.g., a BoNT/A enzymatic domain isoform, a BoNT/B enzymatic domain isoform, a BoNT/C1 enzymatic domain isoform, a BoNT/D enzymatic domain isoform, a BoNT/E enzymatic domain isoform, a BoNT/F enzymatic domain isoform, a BoNT/G enzymatic domain isoform, and a TeNT enzymatic domain isoform. A Clostridial toxin enzymatic domain isoform can function in substantially the same manner as the reference Clostridial toxin enzymatic domain on which the Clostridial toxin enzymatic domain isoform is based, and can be substituted for the reference Clostridial toxin enzymatic domain in any aspect of the present invention.

Another non-limiting examples of a naturally occurring Clostridial toxin enzymatic domain variant is a Clostridial toxin enzymatic domain subtype such as, e.g., an enzymatic domain from subtype BoNT/A1, BoNT/A2, BoNT/A3, BoNT/A4 and BoNT/A5; an enzymatic domain from subtype BoNT/B1, BoNT/B2, BoNT/B bivalent and BoNT/B nonproteolytic; an enzymatic domain from subtype BoNT/C1-1 and BoNT/C1-2; an enzymatic domain from subtype BoNT/E1, BoNT/E2 and BoNT/E3; and an enzymatic domain from subtype BoNT/F1, BoNT/F2, BoNT/F3 and BoNT/F4. A Clostridial toxin enzymatic domain subtype can function in substantially the same manner as the reference Clostridial toxin enzymatic domain on which the Clostridial toxin enzy-

matic domain subtype is based, and can be substituted for the reference Clostridial toxin enzymatic domain in any aspect of the present invention.

As used herein, the term "non-naturally occurring Clostridial toxin enzymatic domain variant" means any Clostridial toxin enzymatic domain produced with the aid of human manipulation, including, without limitation, Clostridial toxin enzymatic domains produced by genetic engineering using random mutagenesis or rational design and Clostridial toxin enzymatic domains produced by chemical synthesis. Non-limiting examples of non-naturally occurring Clostridial toxin enzymatic domain variants include, e.g., conservative Clostridial toxin enzymatic domain variants, non-conservative Clostridial toxin enzymatic domain variants, Clostridial toxin enzymatic domain chimeric variants and active Clostridial toxin enzymatic domain fragments.

As used herein, the term "conservative Clostridial toxin enzymatic domain variant" means a Clostridial toxin enzymatic domain that has at least one amino acid substituted by another amino acid or an amino acid analog that has at least one property similar to that of the original amino acid from the reference Clostridial toxin enzymatic domain sequence (Table 1). Examples of properties include, without limitation, similar size, topography, charge, hydrophobicity, hydrophilicity, lipophilicity, covalent-bonding capacity, hydrogen-bonding capacity, a physicochemical property, of the like, or any combination thereof. A conservative Clostridial toxin enzymatic domain variant can function in substantially the same manner as the reference Clostridial toxin enzymatic domain on which the conservative Clostridial toxin enzymatic domain variant is based, and can be substituted for the reference Clostridial toxin enzymatic domain in any aspect of the present invention. A conservative Clostridial toxin enzymatic domain variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, ten or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids, 50 or more amino acids, 100 or more amino acids, or 200 or more amino acids, from the reference Clostridial toxin enzymatic domain on which the conservative Clostridial toxin enzymatic domain variant is based. A conservative Clostridial toxin enzymatic domain variant can also substitute at least 10 contiguous amino acids, at least 15 contiguous amino acids, at least 20 contiguous amino acids, or at least 25 contiguous amino acids from the reference Clostridial toxin enzymatic domain on which the conservative Clostridial toxin enzymatic domain variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference Clostridial toxin enzymatic domain on which the conservative Clostridial toxin enzymatic domain variant is based. Non-limiting examples of a conservative Clostridial toxin enzymatic domain variant include, e.g., conservative BoNT/A enzymatic domain variants, conservative BoNT/B enzymatic domain variants, conservative BoNT/C1 enzymatic domain variants, conservative BoNT/D enzymatic domain variants, conservative BoNT/E enzymatic domain variants, conservative BoNT/F enzymatic domain variants, conservative BoNT/G enzymatic domain variants, and conservative TeNT enzymatic domain variants.

As used herein, the term "non-conservative Clostridial toxin enzymatic domain variant" means a Clostridial toxin enzymatic domain in which 1) at least one amino acid is deleted from the reference Clostridial toxin enzymatic domain on which the non-conservative Clostridial toxin enzymatic domain variant is based; 2) at least one amino acid added to the reference Clostridial toxin enzymatic domain on which the non-conservative Clostridial toxin enzymatic domain is based; or 3) at least one amino acid is substituted by another amino acid or an amino acid analog that does not share any property similar to that of the original amino acid from the reference Clostridial toxin enzymatic domain sequence (Table 1). A non-conservative Clostridial toxin enzymatic domain variant can function in substantially the same manner as the reference Clostridial toxin enzymatic domain on which the non-conservative Clostridial toxin enzymatic domain variant is based, and can be substituted for the reference Clostridial toxin enzymatic domain in any aspect of the present invention. A non-conservative Clostridial toxin enzymatic domain variant can delete one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, and ten or more amino acids from the reference Clostridial toxin enzymatic domain on which the non-conservative Clostridial toxin enzymatic domain variant is based. A non-conservative Clostridial toxin enzymatic domain variant can add one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, and ten or more amino acids to the reference Clostridial toxin enzymatic domain on which the non-conservative Clostridial toxin enzymatic domain variant is based. A non-conservative Clostridial toxin enzymatic domain variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, ten or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids, 50 or more amino acids, 100 or more amino acids, or 200 or more amino acids from the reference Clostridial toxin enzymatic domain on which the non-conservative Clostridial toxin enzymatic domain variant is based. A non-conservative Clostridial toxin enzymatic domain variant can also substitute at least 10 contiguous amino acids, at least 15 contiguous amino acids, at least 20 contiguous amino acids, or at least 25 contiguous amino acids from the reference Clostridial toxin enzymatic domain on which the non-conservative Clostridial toxin enzymatic domain variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference Clostridial toxin enzymatic domain on which the non-conservative Clostridial toxin enzymatic domain variant is based. Non-limiting examples of a non-conservative Clostridial toxin enzymatic domain variant include, e.g., non-conservative BoNT/A enzymatic domain variants, non-conservative BoNT/B enzymatic domain variants, non-conservative BoNT/C1 enzymatic domain variants, non-conservative BoNT/D enzymatic domain variants, non-conservative BoNT/E enzymatic domain variants, non-conservative BoNT/F enzymatic domain variants, non-conservative BoNT/G enzymatic domain variants, and non-conservative TeNT enzymatic domain variants.

As used herein, the term "Clostridial toxin enzymatic domain chimeric" means a polypeptide comprising at least a portion of a Clostridial toxin enzymatic domain and at least a portion of at least one other polypeptide to form a toxin enzymatic domain with at least one property different from the reference Clostridial toxin enzymatic domains of Table 1, with the proviso that this Clostridial toxin enzymatic domain chimeric is still capable of specifically targeting the core components of the neurotransmitter release apparatus and thus participate in executing the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate. Such Clostridial toxin enzymatic domain chimerics are

described in, e.g., Lance E. Steward et al., Leucine-based Motif and Clostridial Toxins, U.S. Patent Publication 2003/0027752 (Feb. 6, 2003); Lance E. Steward et al., Clostridial Neurotoxin Compositions and Modified Clostridial Neurotoxins, U.S. Patent Publication 2003/0219462 (Nov. 27, 2003); and Lance E. Steward et al., Clostridial Neurotoxin Compositions and Modified Clostridial Neurotoxins, U.S. Patent Publication 2004/0220386 (Nov. 4, 2004), each of which is incorporated by reference in its entirety.

As used herein, the term "active Clostridial toxin enzymatic domain fragment" means any of a variety of Clostridial toxin fragments comprising the enzymatic domain can be useful in aspects of the present invention with the proviso that these enzymatic domain fragments can specifically target the core components of the neurotransmitter release apparatus and thus participate in executing the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate. The enzymatic domains of Clostridial toxins are approximately 420-460 amino acids in length and comprise an enzymatic domain (Table 1). Research has shown that the entire length of a Clostridial toxin enzymatic domain is not necessary for the enzymatic activity of the enzymatic domain. As a non-limiting example, the first eight amino acids of the BoNT/A enzymatic domain (residues 1-8 of SEQ ID NO: 1) are not required for enzymatic activity. As another non-limiting example, the first eight amino acids of the TeNT enzymatic domain (residues 1-8 of SEQ ID NO: 8) are not required for enzymatic activity. Likewise, the carboxyl-terminus of the enzymatic domain is not necessary for activity. As a non-limiting example, the last 32 amino acids of the BoNT/A enzymatic domain (residues 417-448 of SEQ ID NO: 1) are not required for enzymatic activity. As another non-limiting example, the last 31 amino acids of the TeNT enzymatic domain (residues 427-457 of SEQ ID NO: 8) are not required for enzymatic activity. Thus, aspects of this embodiment can include Clostridial toxin enzymatic domains comprising an enzymatic domain having a length of, e.g., at least 350 amino acids, at least 375 amino acids, at least 400 amino acids, at least 425 amino acids and at least 450 amino acids. Other aspects of this embodiment can include Clostridial toxin enzymatic domains comprising an enzymatic domain having a length of, e.g., at most 350 amino acids, at most 375 amino acids, at most 400 amino acids, at most 425 amino acids and at most 450 amino acids.

Any of a variety of sequence alignment methods can be used to determine percent identity of naturally-occurring Clostridial toxin enzymatic domain variants and non-naturally-occurring Clostridial toxin enzymatic domain variants, including, without limitation, global methods, local methods and hybrid methods, such as, e.g., segment approach methods. Protocols to determine percent identity are routine procedures within the scope of one skilled in the art and from the teaching herein.

Global methods align sequences from the beginning to the end of the molecule and determine the best alignment by adding up scores of individual residue pairs and by imposing gap penalties. Non-limiting methods include, e.g., CLUSTAL W, see, e.g., Julie D. Thompson et al., *CLUSTAL W: Improving the Sensitivity of Progressive Multiple Sequence Alignment Through Sequence Weighting, Position-Specific Gap Penalties and Weight Matrix Choice,* 22(22) Nucleic Acids Research 4673-4680 (1994); and iterative refinement, see, e.g., Osamu Gotoh, *Significant Improvement in Accuracy of Multiple Protein Sequence Alignments by Iterative Refinement as Assessed by Reference to Structural Alignments,* 264(4) J. Mol. Biol. 823-838 (1996).

Local methods align sequences by identifying one or more conserved motifs shared by all of the input sequences. Non-limiting methods include, e.g., Match-box, see, e.g., Eric Depiereux and Ernest Feytmans, *Match-Box: A Fundamentally New Algorithm for the Simultaneous Alignment of Several Protein Sequences,* 8(5) CABIOS 501-509 (1992); Gibbs sampling, see, e.g., C. E. Lawrence et al., *Detecting Subtle Sequence Signals: A Gibbs Sampling Strategy for Multiple Alignment,* 262(5131) Science 208-214 (1993); Align-M, see, e.g., Ivo Van Walle et al., *Align-M—A New Algorithm for Multiple Alignment of Highly Divergent Sequences,* 20(9) Bioinformatics, 1428-1435 (2004).

Hybrid methods combine functional aspects of both global and local alignment methods. Non-limiting methods include, e.g., segment-to-segment comparison, see, e.g., Burkhard Morgenstern et al., *Multiple DNA and Protein Sequence Alignment Based On Segment-to-Segment Comparison,* 93(22) Proc. Natl. Acad. Sci. U.S.A. 12098-12103 (1996); T-Coffee, see, e.g., Cédric Notredame et al., *T-Coffee: A Novel Algorithm for Multiple Sequence Alignment,* 302(1) J. Mol. Biol. 205-217 (2000); MUSCLE, see, e.g., Robert C. Edgar, *MUSCLE: Multiple Sequence Alignment With High Score Accuracy and High Throughput,* 32(5) Nucleic Acids Res. 1792-1797 (2004); and DIALIGN-T, see, e.g., Amarendran R Subramanian et al., *DIALIGN-T: An Improved Algorithm for Segment-Based Multiple Sequence Alignment,* 6(1) BMC Bioinformatics 66 (2005).

Thus, in an embodiment, a modified Clostridial toxin disclosed in the present specification comprises a Clostridial toxin enzymatic domain. In an aspect of this embodiment, a Clostridial toxin enzymatic domain comprises a naturally occurring Clostridial toxin enzymatic domain variant, such as, e.g., a Clostridial toxin enzymatic domain isoform or a Clostridial toxin enzymatic domain subtype. In another aspect of this embodiment, a Clostridial toxin enzymatic domain comprises a non-naturally occurring Clostridial toxin enzymatic domain variant, such as, e.g., a conservative Clostridial toxin enzymatic domain variant, a non-conservative Clostridial toxin enzymatic domain variant, a Clostridial toxin chimeric enzymatic domain, an active Clostridial toxin enzymatic domain fragment, or any combination thereof.

In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/A enzymatic domain. In an aspect of this embodiment, a BoNT/A enzymatic domain comprises amino acids 1-448 of SEQ ID NO: 1. In another aspect of this embodiment, a BoNT/A enzymatic domain comprises a naturally occurring BoNT/A enzymatic domain variant, such as, e.g., an enzymatic domain from a BoNT/A isoform or an enzymatic domain from a BoNT/A subtype. In another aspect of this embodiment, a BoNT/A enzymatic domain comprises amino acids 1-448 of a naturally occurring BoNT/A enzymatic domain variant of SEQ ID NO: 1, such as, e.g., amino acids 1-448 of a BoNT/A isoform of SEQ ID NO: 1 or amino acids 1-448 of a BoNT/A subtype of SEQ ID NO: 1. In still another aspect of this embodiment, a BoNT/A enzymatic domain comprises a non-naturally occurring BoNT/A enzymatic domain variant, such as, e.g., a conservative BoNT/A enzymatic domain variant, a non-conservative BoNT/A enzymatic domain variant, a BoNT/A chimeric enzymatic domain, an active BoNT/A enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/A enzymatic domain comprises amino acids 1-448 of a non-naturally occurring BoNT/A enzymatic domain variant of SEQ ID NO: 1, such as, e.g., amino acids 1-448 of a conservative BoNT/A enzymatic domain variant of SEQ ID NO: 1, amino acids 1-448 of a non-conservative BoNT/A enzymatic domain variant of SEQ ID NO: 1, amino

acids 1-448 of an active BoNT/A enzymatic domain fragment of SEQ ID NO: 1, or any combination thereof.

In other aspects of this embodiment, a BoNT/A enzymatic domain comprises a polypeptide having an amino acid identity to amino acids 1-448 of SEQ ID NO: 1 of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%. In yet other aspects of this embodiment, a BoNT/A enzymatic domain comprises a polypeptide having an amino acid identity to amino acids 1-448 of SEQ ID NO: 1 of, e.g., at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95%.

In other aspects of this embodiment, a BoNT/A enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 1-448 of SEQ ID NO: 1; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 1-448 of SEQ ID NO: 1; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 1-448 of SEQ ID NO: 1; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 1-448 of SEQ ID NO: 1; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 1-448 of SEQ ID NO: 1; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 1-448 of SEQ ID NO: 1.

In other aspects of this embodiment, a BoNT/A enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 1-448 of SEQ ID NO: 1; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 1-448 of SEQ ID NO: 1; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 1-448 of SEQ ID NO: 1; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 1-448 of SEQ ID NO: 1; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 1-448 of SEQ ID NO: 1; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 1-448 of SEQ ID NO: 1.

In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/B enzymatic domain. In an aspect of this embodiment, a BoNT/B enzymatic domain comprises amino acids 1-441 of SEQ ID NO: 2. In another aspect of this embodiment, a BoNT/B enzymatic domain comprises a naturally occurring BoNT/B enzymatic domain variant, such as, e.g., an enzymatic domain from a BoNT/B isoform or an enzymatic domain from a BoNT/B subtype. In another aspect of this embodiment, a BoNT/B enzymatic domain comprises amino acids 1-441 of a naturally occurring BoNT/B enzymatic domain variant of SEQ ID NO: 2, such as, e.g., amino acids 1-441 of a BoNT/B isoform of SEQ ID NO: 2 or amino acids 1-441 of a BoNT/B subtype of SEQ ID NO: 2. In still another aspect of this embodiment, a BoNT/B enzymatic domain comprises a non-naturally occurring BoNT/B enzymatic domain variant, such as, e.g., a conservative BoNT/B enzymatic domain variant, a non-conservative BoNT/B enzymatic domain variant, a BoNT/B chimeric enzymatic domain, an active BoNT/B enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/B enzymatic domain comprises amino acids 1-441 of a non-naturally occurring BoNT/B enzymatic domain variant of SEQ ID NO: 2, such as, e.g., amino acids 1-441 of a conservative BoNT/B enzymatic domain variant of SEQ ID NO: 2, amino acids 1-441 of a non-conservative BoNT/B enzymatic domain variant of SEQ ID NO: 2, amino acids 1-441 of an active BoNT/B enzymatic domain fragment of SEQ ID NO: 2, or any combination thereof.

In other aspects of this embodiment, a BoNT/B enzymatic domain comprises a polypeptide having an amino acid identity to amino acids 1-441 of SEQ ID NO: 2 of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%. In yet other aspects of this embodiment, a BoNT/B enzymatic domain comprises a polypeptide having an amino acid identity to amino acids 1-441 of SEQ ID NO: 2 of, e.g., at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95%.

In other aspects of this embodiment, a BoNT/B enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 1-441 of SEQ ID NO: 2; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 1-441 of SEQ ID NO: 2; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 1-441 of SEQ ID NO: 2; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 1-441 of SEQ ID NO: 2; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 1-441 of SEQ ID NO: 2; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 1-441 of SEQ ID NO: 2.

In other aspects of this embodiment, a BoNT/B enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 1-441 of SEQ ID NO: 2; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 1-441 of SEQ ID NO: 2; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 1-441 of SEQ ID NO: 2; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 1-441 of SEQ ID NO: 2; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 1-441 of SEQ ID NO: 2; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 1-441 of SEQ ID NO: 2.

In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/C1 enzymatic domain. In an aspect of this embodiment, a BoNT/C1 enzymatic domain comprises amino acids 1-449 of SEQ ID NO: 3. In another aspect of this embodiment, a BoNT/C1 enzymatic domain comprises a naturally occurring BoNT/C1 enzymatic domain variant, such as, e.g., an enzymatic domain from a BoNT/C1 isoform or an enzymatic domain from a BoNT/C1 subtype. In another aspect of this embodiment, a BoNT/C1 enzymatic domain comprises amino acids 1-449 of a naturally occurring BoNT/C1 enzymatic domain variant of SEQ ID NO: 3, such as, e.g., amino acids 1-449 of a BoNT/C1 isoform of SEQ ID NO: 3 or amino acids 1-449 of a BoNT/C1 subtype of SEQ ID NO: 3. In still another aspect of this embodiment, a BoNT/C1 enzymatic domain comprises a non-naturally occurring BoNT/C1 enzymatic domain variant, such as, e.g., a conservative BoNT/C1 enzymatic domain variant, a non-conservative BoNT/C1 enzymatic domain variant, a BoNT/C1 chimeric enzymatic domain, an active BoNT/C1 enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/C1 enzymatic domain comprises amino acids 1-449 of a non-naturally occurring

BoNT/C1 enzymatic domain variant of SEQ ID NO: 3, such as, e.g., amino acids 1-449 of a conservative BoNT/C1 enzymatic domain variant of SEQ ID NO: 3, amino acids 1-449 of a non-conservative BoNT/C1 enzymatic domain variant of SEQ ID NO: 3, amino acids 1-449 of an active BoNT/C1 enzymatic domain fragment of SEQ ID NO: 3, or any combination thereof.

In other aspects of this embodiment, a BoNT/C1 enzymatic domain comprises a polypeptide having an amino acid identity to amino acids 1-449 of SEQ ID NO: 3 of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%. In yet other aspects of this embodiment, a BoNT/C1 enzymatic domain comprises a polypeptide having an amino acid identity to amino acids 1-449 of SEQ ID NO: 3 of, e.g., at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95%.

In other aspects of this embodiment, a BoNT/C1 enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 1-449 of SEQ ID NO: 3; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 1-449 of SEQ ID NO: 3; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 1-449 of SEQ ID NO: 3; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 1-449 of SEQ ID NO: 3; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 1-449 of SEQ ID NO: 3; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 1-449 of SEQ ID NO: 3.

In other aspects of this embodiment, a BoNT/C1 enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 1-449 of SEQ ID NO: 3; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 1-449 of SEQ ID NO: 3; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 1-449 of SEQ ID NO: 3; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 1-449 of SEQ ID NO: 3; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 1-449 of SEQ ID NO: 3; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 1-449 of SEQ ID NO: 3.

In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/D enzymatic domain. In an aspect of this embodiment, a BoNT/D enzymatic domain comprises amino acids 1-445 of SEQ ID NO: 4. In another aspect of this embodiment, a BoNT/D enzymatic domain comprises a naturally occurring BoNT/D enzymatic domain variant, such as, e.g., an enzymatic domain from a BoNT/D isoform or an enzymatic domain from a BoNT/D subtype. In another aspect of this embodiment, a BoNT/D enzymatic domain comprises amino acids 1-445 of a naturally occurring BoNT/D enzymatic domain variant of SEQ ID NO: 4, such as, e.g., amino acids 1-445 of a BoNT/D isoform of SEQ ID NO: 4 or amino acids 1-445 of a BoNT/D subtype of SEQ ID NO: 4. In still another aspect of this embodiment, a BoNT/D enzymatic domain comprises a non-naturally occurring BoNT/D enzymatic domain variant, such as, e.g., a conservative BoNT/D enzymatic domain variant, a non-conservative BoNT/D enzymatic domain variant, a BoNT/D chimeric enzymatic domain, an active BoNT/D enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/D enzymatic domain comprises amino acids 1-445 of a non-naturally occurring BoNT/D enzymatic domain variant of SEQ ID NO: 4, such as, e.g., amino acids 1-445 of a conservative BoNT/D enzymatic domain variant of SEQ ID NO: 4, amino acids 1-445 of a non-conservative BoNT/D enzymatic domain variant of SEQ ID NO: 4, amino acids 1-445 of an active BoNT/D enzymatic domain fragment of SEQ ID NO: 4, or any combination thereof.

In other aspects of this embodiment, a BoNT/D enzymatic domain comprises a polypeptide having an amino acid identity to amino acids 1-445 of SEQ ID NO: 4 of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%. In yet other aspects of this embodiment, a BoNT/D enzymatic domain comprises a polypeptide having an amino acid identity to amino acids 1-445 of SEQ ID NO: 4 of, e.g., at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95%.

In other aspects of this embodiment, a BoNT/D enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 1-445 of SEQ ID NO: 4; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 1-445 of SEQ ID NO: 4; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 1-445 of SEQ ID NO: 4; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 1-445 of SEQ ID NO: 4; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 1-445 of SEQ ID NO: 4; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 1-445 of SEQ ID NO: 4.

In other aspects of this embodiment, a BoNT/D enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 1-445 of SEQ ID NO: 4; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 1-445 of SEQ ID NO: 4; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 1-445 of SEQ ID NO: 4; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 1-445 of SEQ ID NO: 4; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 1-445 of SEQ ID NO: 4; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 1-445 of SEQ ID NO: 4.

In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/E enzymatic domain. In an aspect of this embodiment, a BoNT/E enzymatic domain comprises amino acids 1-422 of SEQ ID NO: 5. In another aspect of this embodiment, a BoNT/E enzymatic domain comprises a naturally occurring BoNT/E enzymatic domain variant, such as, e.g., an enzymatic domain from a BoNT/E isoform or an enzymatic domain from a BoNT/E subtype. In another aspect of this embodiment, a BoNT/E enzymatic domain comprises amino acids 1-422 of a naturally occurring BoNT/E enzymatic domain variant of SEQ ID NO: 5, such as, e.g., amino acids 1-422 of a BoNT/E isoform of SEQ ID NO: 5 or amino acids 1-422 of a BoNT/E subtype of SEQ ID NO: 5. In still another aspect of this embodiment, a BoNT/E enzymatic domain comprises a non-naturally occurring BoNT/E enzymatic domain variant, such as, e.g., a conservative BoNT/E enzymatic domain variant, a non-conservative BoNT/E enzy-

matic domain variant, a BoNT/E chimeric enzymatic domain, an active BoNT/E enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/E enzymatic domain comprises amino acids 1-422 of a non-naturally occurring BoNT/E enzymatic domain variant of SEQ ID NO: 5, such as, e.g., amino acids 1-422 of a conservative BoNT/E enzymatic domain variant of SEQ ID NO: 5, amino acids 1-422 of a non-conservative BoNT/E enzymatic domain variant of SEQ ID NO: 5, amino acids 1-422 of an active BoNT/E enzymatic domain fragment of SEQ ID NO: 5, or any combination thereof.

In other aspects of this embodiment, a BoNT/E enzymatic domain comprises a polypeptide having an amino acid identity to amino acids 1-422 of SEQ ID NO: 5 of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%. In yet other aspects of this embodiment, a BoNT/E enzymatic domain comprises a polypeptide having an amino acid identity to amino acids 1-422 of SEQ ID NO: 5 of, e.g., at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95%.

In other aspects of this embodiment, a BoNT/E enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 1-422 of SEQ ID NO: 5; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 1-422 of SEQ ID NO: 5; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 1-422 of SEQ ID NO: 5; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 1-422 of SEQ ID NO: 5; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 1-422 of SEQ ID NO: 5; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 1-422 of SEQ ID NO: 5.

In other aspects of this embodiment, a BoNT/E enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 1-422 of SEQ ID NO: 5; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 1-422 of SEQ ID NO: 5; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 1-422 of SEQ ID NO: 5; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 1-422 of SEQ ID NO: 5; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 1-422 of SEQ ID NO: 5; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 1-422 of SEQ ID NO: 5.

In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/F enzymatic domain. In an aspect of this embodiment, a BoNT/F enzymatic domain comprises amino acids 1-439 of SEQ ID NO: 6. In another aspect of this embodiment, a BoNT/F enzymatic domain comprises a naturally occurring BoNT/F enzymatic domain variant, such as, e.g., an enzymatic domain from a BoNT/F isoform or an enzymatic domain from a BoNT/F subtype. In another aspect of this embodiment, a BoNT/F enzymatic domain comprises amino acids 1-439 of a naturally occurring BoNT/F enzymatic domain variant of SEQ ID NO: 6, such as, e.g., amino acids 1-439 of a BoNT/F isoform of SEQ ID NO: 6 or amino acids 1-439 of a BoNT/F subtype of SEQ ID NO: 6. In still another aspect of this embodiment, a BoNT/F enzymatic domain comprises a non-naturally occurring BoNT/F enzymatic domain variant, such as, e.g., a conservative BoNT/F enzymatic domain variant, a non-conservative BoNT/F enzymatic domain variant, a BoNT/F chimeric enzymatic domain, an active BoNT/F enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/F enzymatic domain comprises amino acids 1-439 of a non-naturally occurring BoNT/F enzymatic domain variant of SEQ ID NO: 6, such as, e.g., amino acids 1-439 of a conservative BoNT/F enzymatic domain variant of SEQ ID NO: 6, amino acids 1-439 of a non-conservative BoNT/F enzymatic domain variant of SEQ ID NO: 6, amino acids 1-439 of an active BoNT/F enzymatic domain fragment of SEQ ID NO: 6, or any combination thereof.

In other aspects of this embodiment, a BoNT/F enzymatic domain comprises a polypeptide having an amino acid identity to amino acids 1-439 of SEQ ID NO: 6 of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%. In yet other aspects of this embodiment, a BoNT/F enzymatic domain comprises a polypeptide having an amino acid identity to amino acids 1-439 of SEQ ID NO: 6 of, e.g., at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95%.

In other aspects of this embodiment, a BoNT/F enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 1-439 of SEQ ID NO: 6; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 1-439 of SEQ ID NO: 6; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 1-439 of SEQ ID NO: 6; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 1-439 of SEQ ID NO: 6; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 1-439 of SEQ ID NO: 6; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 1-439 of SEQ ID NO: 6.

In other aspects of this embodiment, a BoNT/F enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 1-439 of SEQ ID NO: 6; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 1-439 of SEQ ID NO: 6; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 1-439 of SEQ ID NO: 6; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 1-439 of SEQ ID NO: 6; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 1-439 of SEQ ID NO: 6; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 1-439 of SEQ ID NO: 6.

In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/G enzymatic domain. In an aspect of this embodiment, a BoNT/G enzymatic domain comprises amino acids 1-446 of SEQ ID NO: 7. In another aspect of this embodiment, a BoNT/G enzymatic domain comprises a naturally occurring BoNT/G enzymatic domain variant, such as, e.g., an enzymatic domain from a BoNT/G isoform or an enzymatic domain from a BoNT/G subtype. In another aspect of this embodiment, a BoNT/G enzymatic domain comprises amino acids 1-446 of a naturally occurring BoNT/G enzymatic domain variant of SEQ ID NO: 7, such as, e.g., amino acids 1-446 of a BoNT/G isoform of SEQ ID NO: 7 or amino acids 1-446 of a BoNT/G subtype of SEQ ID NO: 7. In still

another aspect of this embodiment, a BoNT/G enzymatic domain comprises a non-naturally occurring BoNT/G enzymatic domain variant, such as, e.g., a conservative BoNT/G enzymatic domain variant, a non-conservative BoNT/G enzymatic domain variant, a BoNT/G chimeric enzymatic domain, an active BoNT/G enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/G enzymatic domain comprises amino acids 1-446 of a non-naturally occurring BoNT/G enzymatic domain variant of SEQ ID NO: 7, such as, e.g., amino acids 1-446 of a conservative BoNT/G enzymatic domain variant of SEQ ID NO: 7, amino acids 1-446 of a non-conservative BoNT/G enzymatic domain variant of SEQ ID NO: 7, amino acids 1-446 of an active BoNT/G enzymatic domain fragment of SEQ ID NO: 7, or any combination thereof.

In other aspects of this embodiment, a BoNT/G enzymatic domain comprises a polypeptide having an amino acid identity to amino acids 1-446 of SEQ ID NO: 7 of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%. In yet other aspects of this embodiment, a BoNT/G enzymatic domain comprises a polypeptide having an amino acid identity to amino acids 1-446 of SEQ ID NO: 7 of, e.g., at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95%.

In other aspects of this embodiment, a BoNT/G enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 1-446 of SEQ ID NO: 7; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 1-446 of SEQ ID NO: 7; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 1-446 of SEQ ID NO: 7; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 1-446 of SEQ ID NO: 7; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 1-446 of SEQ ID NO: 7; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 1-446 of SEQ ID NO: 7.

In other aspects of this embodiment, a BoNT/G enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 1-446 of SEQ ID NO: 7; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 1-446 of SEQ ID NO: 7; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 1-446 of SEQ ID NO: 7; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 1-446 of SEQ ID NO: 7; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 1-446 of SEQ ID NO: 7; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 1-446 of SEQ ID NO: 7.

In another embodiment, a Clostridial toxin enzymatic domain comprises a TeNT enzymatic domain. In an aspect of this embodiment, a TeNT enzymatic domain comprises amino acids 1-457 of SEQ ID NO: 8. In another aspect of this embodiment, a TeNT enzymatic domain comprises a naturally occurring TeNT enzymatic domain variant, such as, e.g., an enzymatic domain from a TeNT isoform or an enzymatic domain from a TeNT subtype. In another aspect of this embodiment, a TeNT enzymatic domain comprises amino acids 1-457 of a naturally occurring TeNT enzymatic domain variant of SEQ ID NO: 8, such as, e.g., amino acids 1-457 of a TeNT isoform of SEQ ID NO: 8 or amino acids 1-457 of a TeNT subtype of SEQ ID NO: 8. In still another aspect of this embodiment, a TeNT enzymatic domain comprises a non-naturally occurring TeNT enzymatic domain variant, such as, e.g., a conservative TeNT enzymatic domain variant, a non-conservative TeNT enzymatic domain variant, a TeNT chimeric enzymatic domain, an active TeNT enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a TeNT enzymatic domain comprises amino acids 1-457 of a non-naturally occurring TeNT enzymatic domain variant of SEQ ID NO: 8, such as, e.g., amino acids 1-457 of a conservative TeNT enzymatic domain variant of SEQ ID NO: 8, amino acids 1-457 of a non-conservative TeNT enzymatic domain variant of SEQ ID NO: 8, amino acids 1-457 of an active TeNT enzymatic domain fragment of SEQ ID NO: 8, or any combination thereof.

In other aspects of this embodiment, a TeNT enzymatic domain comprises a polypeptide having an amino acid identity to amino acids 1-457 of SEQ ID NO: 8 of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%. In yet other aspects of this embodiment, a TeNT enzymatic domain comprises a polypeptide having an amino acid identity to amino acids 1-457 of SEQ ID NO: 8 of, e.g., at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95%.

In other aspects of this embodiment, a TeNT enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 1-457 of SEQ ID NO: 8; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 1-457 of SEQ ID NO: 8; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 1-457 of SEQ ID NO: 8; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 1-457 of SEQ ID NO: 8; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 1-457 of SEQ ID NO: 8; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 1-457 of SEQ ID NO: 8.

In other aspects of this embodiment, a TeNT enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 1-457 of SEQ ID NO: 8; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 1-457 of SEQ ID NO: 8; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 1-457 of SEQ ID NO: 8; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 1-457 of SEQ ID NO: 8; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 1-457 of SEQ ID NO: 8; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 1-457 of SEQ ID NO: 8.

In another embodiment, a Clostridial toxin enzymatic domain comprises a BaNT enzymatic domain. In an aspect of this embodiment, a BaNT enzymatic domain comprises amino acids 1-431 of SEQ ID NO: 9. In another aspect of this embodiment, a BaNT enzymatic domain comprises a naturally occurring BaNT enzymatic domain variant, such as, e.g., an enzymatic domain from a BaNT isoform or an enzymatic domain from a BaNT subtype. In another aspect of this embodiment, a BaNT enzymatic domain comprises amino acids 1-431 of a naturally occurring BaNT enzymatic domain

variant of SEQ ID NO: 9, such as, e.g., amino acids 1-431 of a BaNT isoform of SEQ ID NO: 9 or amino acids 1-431 of a BaNT subtype of SEQ ID NO: 9. In still another aspect of this embodiment, a BaNT enzymatic domain comprises a non-naturally occurring BaNT enzymatic domain variant, such as, e.g., a conservative BaNT enzymatic domain variant, a non-conservative BaNT enzymatic domain variant, a BaNT chimeric enzymatic domain, an active BaNT enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BaNT enzymatic domain comprises amino acids 1-431 of a non-naturally occurring BaNT enzymatic domain variant of SEQ ID NO: 9, such as, e.g., amino acids 1-431 of a conservative BaNT enzymatic domain variant of SEQ ID NO: 9, amino acids 1-431 of a non-conservative BaNT enzymatic domain variant of SEQ ID NO: 9, amino acids 1-431 of an active BaNT enzymatic domain fragment of SEQ ID NO: 9, or any combination thereof.

In other aspects of this embodiment, a BaNT enzymatic domain comprises a polypeptide having an amino acid identity to amino acids 1-431 of SEQ ID NO: 9 of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%. In yet other aspects of this embodiment, a BaNT enzymatic domain comprises a polypeptide having an amino acid identity to amino acids 1-431 of SEQ ID NO: 9 of, e.g., at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95%.

In other aspects of this embodiment, a BaNT enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 1-431 of SEQ ID NO: 9; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 1-431 of SEQ ID NO: 9; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 1-431 of SEQ ID NO: 9; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 1-431 of SEQ ID NO: 9; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 1-431 of SEQ ID NO: 9; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 1-431 of SEQ ID NO: 9.

In other aspects of this embodiment, a BaNT enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 1-431 of SEQ ID NO: 9; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 1-431 of SEQ ID NO: 9; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 1-431 of SEQ ID NO: 9; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 1-431 of SEQ ID NO: 9; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 1-431 of SEQ ID NO: 9; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 1-431 of SEQ ID NO: 9.

In another embodiment, a Clostridial toxin enzymatic domain comprises a BuNT enzymatic domain. In an aspect of this embodiment, a BuNT enzymatic domain comprises amino acids 1-422 of SEQ ID NO: 10. In another aspect of this embodiment, a BuNT enzymatic domain comprises a naturally occurring BuNT enzymatic domain variant, such as, e.g., an enzymatic domain from a BuNT isoform or an enzymatic domain from a BuNT subtype. In another aspect of this embodiment, a BuNT enzymatic domain comprises amino acids 1-422 of a naturally occurring BuNT enzymatic domain variant of SEQ ID NO: 10, such as, e.g., amino acids 1-422 of a BuNT isoform of SEQ ID NO: 10 or amino acids 1-422 of a BuNT subtype of SEQ ID NO: 10. In still another aspect of this embodiment, a BuNT enzymatic domain comprises a non-naturally occurring BuNT enzymatic domain variant, such as, e.g., a conservative BuNT enzymatic domain variant, a non-conservative BuNT enzymatic domain variant, a BuNT chimeric enzymatic domain, an active BuNT enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BuNT enzymatic domain comprises amino acids 1-422 of a non-naturally occurring BuNT enzymatic domain variant of SEQ ID NO: 10, such as, e.g., amino acids 1-422 of a conservative BuNT enzymatic domain variant of SEQ ID NO: 10, amino acids 1-422 of a non-conservative BuNT enzymatic domain variant of SEQ ID NO: 10, amino acids 1-422 of an active BuNT enzymatic domain fragment of SEQ ID NO: 10, or any combination thereof.

In other aspects of this embodiment, a BuNT enzymatic domain comprises a polypeptide having an amino acid identity to amino acids 1-422 of SEQ ID NO: 10 of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%. In yet other aspects of this embodiment, a BuNT enzymatic domain comprises a polypeptide having an amino acid identity to amino acids 1-422 of SEQ ID NO: 10 of, e.g., at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95%.

In other aspects of this embodiment, a BuNT enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 1-422 of SEQ ID NO: 1; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 1-422 of SEQ ID NO: 10; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 1-422 of SEQ ID NO: 10; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 1-422 of SEQ ID NO: 10; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 1-422 of SEQ ID NO: 10; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 1-422 of SEQ ID NO: 10.

In other aspects of this embodiment, a BuNT enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-422 of SEQ ID NO: 10. In other aspects of this embodiment, a BuNT enzymatic domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 1-422 of SEQ ID NO: 10. In yet other aspects of this embodiment, a BuNT enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-422 of SEQ ID NO: 10. In other aspects of this embodiment, a BuNT enzymatic domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid deletions relative to amino acids 1-422 of SEQ ID NO: 10. In still other aspects of this embodiment, a BuNT enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-422 of SEQ ID NO: 10. In other aspects of this embodiment, a BuNT enzymatic domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20,

30, 40, 50, 100 or 200 contiguous amino acid additions relative to amino acids 1-422 of SEQ ID NO: 10.

The "translocation domain" comprises a portion of a Clostridial neurotoxin heavy chain having a translocation activity. By "translocation" is meant the ability to facilitate the transport of a polypeptide through a vesicular membrane, thereby exposing some or all of the polypeptide to the cytoplasm. In the various botulinum neurotoxins translocation is thought to involve an allosteric conformational change of the heavy chain caused by a decrease in pH within the endosome. This conformational change appears to involve and be mediated by the N terminal half of the heavy chain and to result in the formation of pores in the vesicular membrane; this change permits the movement of the proteolytic light chain from within the endosomal vesicle into the cytoplasm. See e.g., Lacy, et al., *Nature Struct. Biol.* 5:898-902 (October 1998).

The amino acid sequence of the translocation-mediating portion of the botulinum neurotoxin heavy chain is known to those of skill in the art; additionally, those amino acid residues within this portion that are known to be essential for conferring the translocation activity are also known. It would therefore be well within the ability of one of ordinary skill in the art, for example, to employ the naturally occurring N-terminal peptide half of the heavy chain of any of the various *Clostridium tetanus* or *Clostridium botulinum* neurotoxin subtypes as a translocation domain, or to design an analogous translocation domain by aligning the primary sequences of the N-terminal halves of the various heavy chains and selecting a consensus primary translocation sequence based on conserved amino acid, polarity, steric and hydrophobicity characteristics between the sequences.

In another aspect of the invention, a modified Clostridial toxin comprises, in part, a Clostridial toxin translocation domain. As used herein, the term "Clostridial toxin translocation domain" means any Clostridial toxin polypeptide that can execute the translocation step of the intoxication process that mediates Clostridial toxin light chain translocation. Thus, a Clostridial toxin translocation domain facilitates the movement of a Clostridial toxin light chain across a membrane and encompasses the movement of a Clostridial toxin light chain through the membrane an intracellular vesicle into the cytoplasm of a cell. Non-limiting examples of a Clostridial toxin translocation domain include, e.g., a BoNT/A translocation domain, a BoNT/B translocation domain, a BoNT/C1 translocation domain, a BoNT/D translocation domain, a BoNT/E translocation domain, a BoNT/F translocation domain, a BoNT/G translocation domain, a TeNT translocation domain, a BaNT translocation domain, and a BuNT translocation domain. Other non-limiting examples of a Clostridial toxin translocation domain include, e.g., amino acids 449-873 of SEQ ID NO: 1, amino acids 442-860 of SEQ ID NO: 2, amino acids 450-868 of SEQ ID NO: 3, amino acids 446-864 of SEQ ID NO: 4, amino acids 423-847 of SEQ ID NO: 5, amino acids 440-866 of SEQ ID NO: 6, amino acids 447-865 of SEQ ID NO: 7, amino acids 458-881 of SEQ ID NO: 8, amino acids 432-857 of SEQ ID NO: 9, and amino acids 423-847 of SEQ ID NO: 10.

A Clostridial toxin translocation domain includes, without limitation, naturally occurring Clostridial toxin translocation domain variants, such as, e.g., Clostridial toxin translocation domain isoforms and Clostridial toxin translocation domain subtypes; non-naturally occurring Clostridial toxin translocation domain variants, such as, e.g., conservative Clostridial toxin translocation domain variants, non-conservative Clostridial toxin translocation domain variants, Clostridial toxin translocation domain chimerics, active Clostridial toxin translocation domain fragments thereof, or any combination thereof.

As used herein, the term "Clostridial toxin translocation domain variant," whether naturally-occurring or non-naturally-occurring, means a Clostridial toxin translocation domain that has at least one amino acid change from the corresponding region of the disclosed reference sequences (Table 1) and can be described in percent identity to the corresponding region of that reference sequence. Unless expressly indicated, Clostridial toxin translocation domain variants useful to practice disclosed embodiments are variants that execute the translocation step of the intoxication process that mediates Clostridial toxin light chain translocation. As non-limiting examples, a BoNT/A translocation domain variant comprising amino acids 449-873 of SEQ ID NO: 1 will have at least one amino acid difference, such as, e.g., an amino acid substitution, deletion or addition, as compared to the amino acid region 449-873 of SEQ ID NO: 1; a BoNT/B translocation domain variant comprising amino acids 442-860 of SEQ ID NO: 2 will have at least one amino acid difference, such as, e.g., an amino acid substitution, deletion or addition, as compared to the amino acid region 442-860 of SEQ ID NO: 2; a BoNT/C1 translocation domain variant comprising amino acids 450-868 of SEQ ID NO: 3 will have at least one amino acid difference, such as, e.g., an amino acid substitution, deletion or addition, as compared to the amino acid region 450-868 of SEQ ID NO: 3; a BoNT/D translocation domain variant comprising amino acids 446-864 of SEQ ID NO: 4 will have at least one amino acid difference, such as, e.g., an amino acid substitution, deletion or addition, as compared to the amino acid region 446-864 of SEQ ID NO: 4; a BoNT/E translocation domain variant comprising amino acids 423-847 of SEQ ID NO: 5 will have at least one amino acid difference, such as, e.g., an amino acid substitution, deletion or addition, as compared to the amino acid region 423-847 of SEQ ID NO: 5; a BoNT/F translocation domain variant comprising amino acids 440-866 of SEQ ID NO: 6 will have at least one amino acid difference, such as, e.g., an amino acid substitution, deletion or addition, as compared to the amino acid region 440-866 of SEQ ID NO: 6; a BoNT/G translocation domain variant comprising amino acids 447-865 of SEQ ID NO: 7 will have at least one amino acid difference, such as, e.g., an amino acid substitution, deletion or addition, as compared to the amino acid region 447-865 of SEQ ID NO: 7; a TeNT translocation domain variant comprising amino acids 458-881 of SEQ ID NO: 8 will have at least one amino acid difference, such as, e.g., an amino acid substitution, deletion or addition, as compared to the amino acid region 458-881 of SEQ ID NO: 8; a BaNT translocation domain variant comprising amino acids 432-857 of SEQ ID NO: 9 will have at least one amino acid difference, such as, e.g., an amino acid substitution, deletion or addition, as compared to the amino acid region 432-857 of SEQ ID NO: 9; and a BuNT translocation domain variant comprising amino acids 423-847 of SEQ ID NO: 10 will have at least one amino acid difference, such as, e.g., an amino acid substitution, deletion or addition, as compared to the amino acid region 423-847 of SEQ ID NO: 10.

It is recognized by those of skill in the art that within each serotype of Clostridial toxin there can be naturally occurring Clostridial toxin translocation domain variants that differ somewhat in their amino acid sequence, and also in the nucleic acids encoding these proteins. For example, there are presently five BoNT/A subtypes, BoNT/A1, BoNT/A2, BoNT/A3, BoNT/A4, and BoNT/A5, with specific translocation domain subtypes showing approximately 87% amino

acid identity when compared to another BoNT/A translocation domain subtype. As used herein, the term "naturally occurring Clostridial toxin translocation domain variant" means any Clostridial toxin translocation domain produced by a naturally-occurring process, including, without limitation, Clostridial toxin translocation domain isoforms produced from alternatively-spliced transcripts, Clostridial toxin translocation domain isoforms produced by spontaneous mutation and Clostridial toxin translocation domain subtypes. A naturally occurring Clostridial toxin translocation domain variant can function in substantially the same manner as the reference Clostridial toxin translocation domain on which the naturally occurring Clostridial toxin translocation domain variant is based, and can be substituted for the reference Clostridial toxin translocation domain in any aspect of the present invention. A naturally occurring Clostridial toxin translocation domain variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, ten or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids, 50 or more amino acids or 100 or more amino acids from the reference Clostridial toxin translocation domain on which the naturally occurring Clostridial toxin translocation domain variant is based. A naturally occurring Clostridial toxin translocation domain variant can also substitute at least 10 contiguous amino acids, at least 15 contiguous amino acids, at least 20 contiguous amino acids, or at least 25 contiguous amino acids from the reference Clostridial toxin translocation domain on which the naturally occurring Clostridial toxin translocation domain variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference Clostridial toxin translocation domain on which the naturally occurring Clostridial toxin translocation domain variant is based.

A non-limiting examples of a naturally occurring Clostridial toxin translocation domain variant is a Clostridial toxin translocation domain isoform such as, e.g., a BoNT/A translocation domain isoform, a BoNT/B translocation domain isoform, a BoNT/C1 translocation domain isoform, a BoNT/D translocation domain isoform, a BoNT/E translocation domain isoform, a BoNT/F translocation domain isoform, a BoNT/G translocation domain isoform, a TeNT translocation domain isoform, a BaNT translocation domain isoform, and a BuNT translocation domain isoform. A Clostridial toxin translocation domain isoform can function in substantially the same manner as the reference Clostridial toxin translocation domain on which the Clostridial toxin translocation domain isoform is based, and can be substituted for the reference Clostridial toxin translocation domain in any aspect of the present invention.

Another non-limiting examples of a naturally occurring Clostridial toxin translocation domain variant is a Clostridial toxin translocation domain subtype such as, e.g., a translocation domain from subtype BoNT/A1, BoNT/A2, BoNT/A3, BoNT/A4, and BoNT/A5; a translocation domain from subtype BoNT/B1, BoNT/B2, BoNT/B bivalent and BoNT/B nonproteolytic; a translocation domain from subtype BoNT/C1-1 and BoNT/C1-2; a translocation domain from subtype BoNT/E1, BoNT/E2 and BoNT/E3; and a translocation domain from subtype BoNT/F1, BoNT/F2, BoNT/F3 and BoNT/F4. A Clostridial toxin translocation domain subtype can function in substantially the same manner as the reference Clostridial toxin translocation domain on which the Clostridial toxin translocation domain subtype is based, and can be substituted for the reference Clostridial toxin translocation domain in any aspect of the present invention.

As used herein, the term "non-naturally occurring Clostridial toxin translocation domain variant" means any Clostridial toxin translocation domain produced with the aid of human manipulation, including, without limitation, Clostridial toxin translocation domains produced by genetic engineering using random mutagenesis or rational design and Clostridial toxin translocation domains produced by chemical synthesis. Non-limiting examples of non-naturally occurring Clostridial toxin translocation domain variants include, e.g., conservative Clostridial toxin translocation domain variants, non-conservative Clostridial toxin translocation domain variants, Clostridial toxin translocation domain chimeric variants and active Clostridial toxin translocation domain fragments.

As used herein, the term "conservative Clostridial toxin translocation domain variant" means a Clostridial toxin translocation domain that has at least one amino acid substituted by another amino acid or an amino acid analog that has at least one property similar to that of the original amino acid from the reference Clostridial toxin translocation domain sequence (Table 1). Examples of properties include, without limitation, similar size, topography, charge, hydrophobicity, hydrophilicity, lipophilicity, covalent-bonding capacity, hydrogen-bonding capacity, a physicochemical property, of the like, or any combination thereof. A conservative Clostridial toxin translocation domain variant can function in substantially the same manner as the reference Clostridial toxin translocation domain on which the conservative Clostridial toxin translocation domain variant is based, and can be substituted for the reference Clostridial toxin translocation domain in any aspect of the present invention. A conservative Clostridial toxin translocation domain variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, ten or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids, 50 or more amino acids, 100 or more amino acids, or 200 or more amino acids from the reference Clostridial toxin translocation domain on which the conservative Clostridial toxin translocation domain variant is based. A conservative Clostridial toxin translocation domain variant can also substitute at least 10 contiguous amino acids, at least 15 contiguous amino acids, at least 20 contiguous amino acids, or at least 25 contiguous amino acids from the reference Clostridial toxin translocation domain on which the conservative Clostridial toxin translocation domain variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference Clostridial toxin translocation domain on which the conservative Clostridial toxin translocation domain variant is based. Non-limiting examples of a conservative Clostridial toxin translocation domain variant include, e.g., conservative BoNT/A translocation domain variants, conservative BoNT/B translocation domain variants, conservative BoNT/C1 translocation domain variants, conservative BoNT/D translocation domain variants, conservative BoNT/E translocation domain variants, conservative BoNT/F translocation domain variants, conservative BoNT/G translocation domain variants, conservative TeNT translocation domain variants, conservative BaNT translocation domain variants, and conservative BuNT translocation domain variants.

As used herein, the term "non-conservative Clostridial toxin translocation domain variant" means a Clostridial toxin translocation domain in which 1) at least one amino acid is deleted from the reference Clostridial toxin translocation

domain on which the non-conservative Clostridial toxin translocation domain variant is based; 2) at least one amino acid added to the reference Clostridial toxin translocation domain on which the non-conservative Clostridial toxin translocation domain is based; or 3) at least one amino acid is substituted by another amino acid or an amino acid analog that does not share any property similar to that of the original amino acid from the reference Clostridial toxin translocation domain sequence (Table 1). A non-conservative Clostridial toxin translocation domain variant can function in substantially the same manner as the reference Clostridial toxin translocation domain on which the non-conservative Clostridial toxin translocation domain variant is based, and can be substituted for the reference Clostridial toxin translocation domain in any aspect of the present invention. A non-conservative Clostridial toxin translocation domain variant can delete one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, and ten or more amino acids from the reference Clostridial toxin translocation domain on which the non-conservative Clostridial toxin translocation domain variant is based. A non-conservative Clostridial toxin translocation domain variant can add one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, and ten or more amino acids to the reference Clostridial toxin translocation domain on which the non-conservative Clostridial toxin translocation domain variant is based. A non-conservative Clostridial toxin translocation domain variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, ten or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids, 50 or more amino acids, 100 or more amino acids, or 200 or more amino acids from the reference Clostridial toxin translocation domain on which the non-conservative Clostridial toxin translocation domain variant is based. A non-conservative Clostridial toxin translocation domain variant can also substitute at least 10 contiguous amino acids, at least 15 contiguous amino acids, at least 20 contiguous amino acids, or at least 25 contiguous amino acids from the reference Clostridial toxin translocation domain on which the non-conservative Clostridial toxin translocation domain variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference Clostridial toxin translocation domain on which the non-conservative Clostridial toxin translocation domain variant is based. Non-limiting examples of a non-conservative Clostridial toxin translocation domain variant include, e.g., non-conservative BoNT/A translocation domain variants, non-conservative BoNT/B translocation domain variants, non-conservative BoNT/C1 translocation domain variants, non-conservative BoNT/D translocation domain variants, non-conservative BoNT/E translocation domain variants, non-conservative BoNT/F translocation domain variants, non-conservative BoNT/G translocation domain variants, and non-conservative TeNT translocation domain variants, non-conservative BaNT translocation domain variants, and non-conservative BuNT translocation domain variants.

As used herein, the term "Clostridial toxin translocation domain chimeric" means a polypeptide comprising at least a portion of a Clostridial toxin translocation domain and at least a portion of at least one other polypeptide to form a toxin translocation domain with at least one property different from the reference Clostridial toxin translocation domains of Table 1, with the proviso that this Clostridial toxin translocation domain chimeric is still capable of specifically targeting the core components of the neurotransmitter release apparatus and thus participate in executing the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate.

As used herein, the term "active Clostridial toxin translocation domain fragment" means any of a variety of Clostridial toxin fragments comprising the translocation domain can be useful in aspects of the present invention with the proviso that these active fragments can facilitate the release of the LC from intracellular vesicles into the cytoplasm of the target cell and thus participate in executing the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate. The translocation domains from the heavy chains of Clostridial toxins are approximately 410-430 amino acids in length and comprise a translocation domain (Table 1). Research has shown that the entire length of a translocation domain from a Clostridial toxin heavy chain is not necessary for the translocating activity of the translocation domain. Thus, aspects of this embodiment can include Clostridial toxin translocation domains comprising a translocation domain having a length of, e.g., at least 350 amino acids, at least 375 amino acids, at least 400 amino acids and at least 425 amino acids. Other aspects of this embodiment can include Clostridial toxin translocation domains comprising translocation domain having a length of, e.g., at most 350 amino acids, at most 375 amino acids, at most 400 amino acids and at most 425 amino acids.

Any of a variety of sequence alignment methods can be used to determine percent identity of naturally-occurring Clostridial toxin translocation domain variants and non-naturally-occurring Clostridial toxin translocation domain variants, including, without limitation, global methods, local methods and hybrid methods, such as, e.g., segment approach methods. Protocols to determine percent identity are routine procedures within the scope of one skilled in the art and from the teaching herein.

Thus, in an embodiment, a modified Clostridial toxin disclosed in the present specification comprises a Clostridial toxin translocation domain. In an aspect of this embodiment, a Clostridial toxin translocation domain comprises a naturally occurring Clostridial toxin translocation domain variant, such as, e.g., a Clostridial toxin translocation domain isoform or a Clostridial toxin translocation domain subtype. In another aspect of this embodiment, a Clostridial toxin translocation domain comprises a non-naturally occurring Clostridial toxin translocation domain variant, such as, e.g., a conservative Clostridial toxin translocation domain variant, a non-conservative Clostridial toxin translocation domain variant, a Clostridial toxin chimeric translocation domain, an active Clostridial toxin translocation domain fragment, or any combination thereof.

In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/A translocation domain. In an aspect of this embodiment, a BoNT/A translocation domain comprises amino acids 449-873 of SEQ ID NO: 1. In another aspect of this embodiment, a BoNT/A translocation domain comprises a naturally occurring BoNT/A translocation domain variant, such as, e.g., a translocation domain from a BoNT/A isoform or a translocation domain from a BoNT/A subtype. In another aspect of this embodiment, a BoNT/A translocation domain comprises amino acids 449-873 of a naturally occurring BoNT/A translocation domain variant of SEQ ID NO: 1, such as, e.g., amino acids 449-873 of a BoNT/A isoform of SEQ ID NO: 1 or amino acids 449-873 of a BoNT/A subtype of SEQ ID NO: 1. In still another aspect of this embodiment, a BoNT/A translocation domain comprises a non-naturally occurring BoNT/A translocation domain

variant, such as, e.g., a conservative BoNT/A translocation domain variant, a non-conservative BoNT/A translocation domain variant, a BoNT/A chimeric translocation domain, an active BoNT/A translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/A translocation domain comprises amino acids 449-873 of a non-naturally occurring BoNT/A translocation domain variant of SEQ ID NO: 1, such as, e.g., amino acids 449-873 of a conservative BoNT/A translocation domain variant of SEQ ID NO: 1, amino acids 449-873 of a non-conservative BoNT/A translocation domain variant of SEQ ID NO: 1, amino acids 449-873 of an active BoNT/A translocation domain fragment of SEQ ID NO: 1, or any combination thereof.

In other aspects of this embodiment, a BoNT/A translocation domain comprises a polypeptide having an amino acid identity to amino acids 449-873 of SEQ ID NO: 1 of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%. In yet other aspects of this embodiment, a BoNT/A translocation domain comprises a polypeptide having an amino acid identity to amino acids 449-873 of SEQ ID NO: 1 of, e.g., at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95%.

In other aspects of this embodiment, a BoNT/A translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 449-873 of SEQ ID NO: 1; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 449-873 of SEQ ID NO: 1; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 449-873 of SEQ ID NO: 1; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 449-873 of SEQ ID NO: 1; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 449-873 of SEQ ID NO: 1; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 449-873 of SEQ ID NO: 1.

In other aspects of this embodiment, a BoNT/A translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 449-873 of SEQ ID NO: 1; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100 or 200 contiguous amino acid substitutions relative to amino acids 449-873 of SEQ ID NO: 1; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 449-873 of SEQ ID NO: 1; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 449-873 of SEQ ID NO: 1; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 449-873 of SEQ ID NO: 1; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 449-873 of SEQ ID NO: 1.

In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/B translocation domain. In an aspect of this embodiment, a BoNT/B translocation domain comprises amino acids 442-860 of SEQ ID NO: 2. In another aspect of this embodiment, a BoNT/B translocation domain comprises a naturally occurring BoNT/B translocation domain variant, such as, e.g., a translocation domain from a BoNT/B isoform or a translocation domain from a BoNT/B subtype. In another aspect of this embodiment, a BoNT/B translocation domain comprises amino acids 442-860 of a naturally occurring BoNT/B translocation domain variant of SEQ ID NO: 2, such as, e.g., amino acids 442-860 of a BoNT/B isoform of SEQ ID NO: 2 or amino acids 442-860 of a BoNT/B subtype of SEQ ID NO: 2. In still another aspect of this embodiment, a BoNT/B translocation domain comprises a non-naturally occurring BoNT/B translocation domain variant, such as, e.g., a conservative BoNT/B translocation domain variant, a non-conservative BoNT/B translocation domain variant, a BoNT/B chimeric translocation domain, an active BoNT/B translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/B translocation domain comprises amino acids 442-860 of a non-naturally occurring BoNT/B translocation domain variant of SEQ ID NO: 2, such as, e.g., amino acids 442-860 of a conservative BoNT/B translocation domain variant of SEQ ID NO: 2, amino acids 442-860 of a non-conservative BoNT/B translocation domain variant of SEQ ID NO: 2, amino acids 442-860 of an active BoNT/B translocation domain fragment of SEQ ID NO: 2, or any combination thereof.

In other aspects of this embodiment, a BoNT/B translocation domain comprises a polypeptide having an amino acid identity to amino acids 442-860 of SEQ ID NO: 2 of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%. In yet other aspects of this embodiment, a BoNT/B translocation domain comprises a polypeptide having an amino acid identity to amino acids 442-860 of SEQ ID NO: 2 of, e.g., at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95%.

In other aspects of this embodiment, a BoNT/B translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 442-860 of SEQ ID NO: 2; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100 or 200 non-contiguous amino acid substitutions relative to amino acids 442-860 of SEQ ID NO: 2; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 442-860 of SEQ ID NO: 2; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 442-860 of SEQ ID NO: 2; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 442-860 of SEQ ID NO: 2; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 442-860 of SEQ ID NO: 2.

In other aspects of this embodiment, a BoNT/B translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 442-860 of SEQ ID NO: 2; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 442-860 of SEQ ID NO: 2; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 442-860 of SEQ ID NO: 2; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 442-860 of SEQ ID NO: 2; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 442-860 of SEQ ID NO: 2; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 442-860 of SEQ ID NO: 2.

In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/C1 translocation domain. In an aspect of this embodiment, a BoNT/C1 translocation domain comprises amino acids 450-868 of SEQ ID NO: 3. In another aspect of this embodiment, a BoNT/C1 translocation domain comprises a naturally occurring BoNT/C1 translocation

domain variant, such as, e.g., a translocation domain from a BoNT/C1 isoform or a translocation domain from a BoNT/C1 subtype. In another aspect of this embodiment, a BoNT/C1 translocation domain comprises amino acids 450-868 of a naturally occurring BoNT/C1 translocation domain variant of SEQ ID NO: 3, such as, e.g., amino acids 450-868 of a BoNT/C1 isoform of SEQ ID NO: 3 or amino acids 450-868 of a BoNT/C1 subtype of SEQ ID NO: 3. In still another aspect of this embodiment, a BoNT/C1 translocation domain comprises a non-naturally occurring BoNT/C1 translocation domain variant, such as, e.g., a conservative BoNT/C1 translocation domain variant, a non-conservative BoNT/C1 translocation domain variant, a BoNT/C1 chimeric translocation domain, an active BoNT/C1 translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/C1 translocation domain comprises amino acids 450-868 of a non-naturally occurring BoNT/C1 translocation domain variant of SEQ ID NO: 3, such as, e.g., amino acids 450-868 of a conservative BoNT/C1 translocation domain variant of SEQ ID NO: 3, amino acids 450-868 of a non-conservative BoNT/C1 translocation domain variant of SEQ ID NO: 3, amino acids 450-868 of an active BoNT/C1 translocation domain fragment of SEQ ID NO: 3, or any combination thereof.

In other aspects of this embodiment, a BoNT/C1 translocation domain comprises a polypeptide having an amino acid identity to amino acids 450-868 of SEQ ID NO: 3 of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%. In yet other aspects of this embodiment, a BoNT/C1 translocation domain comprises a polypeptide having an amino acid identity to amino acids 450-868 of SEQ ID NO: 3 of, e.g., at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95%.

In other aspects of this embodiment, a BoNT/C1 translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 450-868 of SEQ ID NO: 3; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 450-868 of SEQ ID NO: 3; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 450-868 of SEQ ID NO: 3; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 450-868 of SEQ ID NO: 3; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 450-868 of SEQ ID NO: 3; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 450-868 of SEQ ID NO: 3.

In other aspects of this embodiment, a BoNT/C1 translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 450-868 of SEQ ID NO: 3; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 450-868 of SEQ ID NO: 3; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 450-868 of SEQ ID NO: 3; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 450-868 of SEQ ID NO: 3; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 450-868 of SEQ ID NO: 3; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 450-868 of SEQ ID NO: 3.

In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/D translocation domain. In an aspect of this embodiment, a BoNT/D translocation domain comprises amino acids 446-864 of SEQ ID NO: 4. In another aspect of this embodiment, a BoNT/D translocation domain comprises a naturally occurring BoNT/D translocation domain variant, such as, e.g., a translocation domain from a BoNT/D isoform or a translocation domain from a BoNT/D subtype. In another aspect of this embodiment, a BoNT/D translocation domain comprises amino acids 446-864 of a naturally occurring BoNT/D translocation domain variant of SEQ ID NO: 4, such as, e.g., amino acids 446-864 of a BoNT/D isoform of SEQ ID NO: 4 or amino acids 446-864 of a BoNT/D subtype of SEQ ID NO: 4. In still another aspect of this embodiment, a BoNT/D translocation domain comprises a non-naturally occurring BoNT/D translocation domain variant, such as, e.g., a conservative BoNT/D translocation domain variant, a non-conservative BoNT/D translocation domain variant, a BoNT/D chimeric translocation domain, an active BoNT/D translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/D translocation domain comprises amino acids 446-864 of a non-naturally occurring BoNT/D translocation domain variant of SEQ ID NO: 4, such as, e.g., amino acids 446-864 of a conservative BoNT/D translocation domain variant of SEQ ID NO: 4, amino acids 446-864 of a non-conservative BoNT/D translocation domain variant of SEQ ID NO: 4, amino acids 446-864 of an active BoNT/D translocation domain fragment of SEQ ID NO: 4, or any combination thereof.

In other aspects of this embodiment, a BoNT/D translocation domain comprises a polypeptide having an amino acid identity to amino acids 446-864 of SEQ ID NO: 4 of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%. In yet other aspects of this embodiment, a BoNT/D translocation domain comprises a polypeptide having an amino acid identity to amino acids 446-864 of SEQ ID NO: 4 of, e.g., at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95%.

In other aspects of this embodiment, a BoNT/D translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 446-864 of SEQ ID NO: 4; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 446-864 of SEQ ID NO: 4; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 446-864 of SEQ ID NO: 4; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 446-864 of SEQ ID NO: 4; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 446-864 of SEQ ID NO: 4; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 446-864 of SEQ ID NO: 4.

In other aspects of this embodiment, a BoNT/D translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 446-864 of SEQ ID NO: 4; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 446-864 of SEQ ID NO: 4; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 446-864 of SEQ ID NO: 4; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 446-864 of SEQ ID NO: 4;

at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 446-864 of SEQ ID NO: 4; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 446-864 of SEQ ID NO: 4.

In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/E translocation domain. In an aspect of this embodiment, a BoNT/E translocation domain comprises amino acids 423-847 of SEQ ID NO: 5. In another aspect of this embodiment, a BoNT/E translocation domain comprises a naturally occurring BoNT/E translocation domain variant, such as, e.g., a translocation domain from a BoNT/E isoform or a translocation domain from a BoNT/E subtype. In another aspect of this embodiment, a BoNT/E translocation domain comprises amino acids 423-847 of a naturally occurring BoNT/E translocation domain variant of SEQ ID NO: 5, such as, e.g., amino acids 423-847 of a BoNT/E isoform of SEQ ID NO: 5 or amino acids 423-847 of a BoNT/E subtype of SEQ ID NO: 5. In still another aspect of this embodiment, a BoNT/E translocation domain comprises a non-naturally occurring BoNT/E translocation domain variant, such as, e.g., a conservative BoNT/E translocation domain variant, a non-conservative BoNT/E translocation domain variant, a BoNT/E chimeric translocation domain, an active BoNT/E translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/E translocation domain comprises amino acids 423-847 of a non-naturally occurring BoNT/E translocation domain variant of SEQ ID NO: 5, such as, e.g., amino acids 423-847 of a conservative BoNT/E translocation domain variant of SEQ ID NO: 5, amino acids 423-847 of a non-conservative BoNT/E translocation domain variant of SEQ ID NO: 5, amino acids 423-847 of an active BoNT/E translocation domain fragment of SEQ ID NO: 5, or any combination thereof.

In other aspects of this embodiment, a BoNT/E translocation domain comprises a polypeptide having an amino acid identity to amino acids 423-847 of SEQ ID NO: 5 of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%. In yet other aspects of this embodiment, a BoNT/E translocation domain comprises a polypeptide having an amino acid identity to amino acids 423-847 of SEQ ID NO: 5 of, e.g., at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95%.

In other aspects of this embodiment, a BoNT/E translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 423-847 of SEQ ID NO: 5; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 423-847 of SEQ ID NO: 5; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 423-847 of SEQ ID NO: 5; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 423-847 of SEQ ID NO: 5; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 423-847 of SEQ ID NO: 5; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 423-847 of SEQ ID NO: 5.

In other aspects of this embodiment, a BoNT/E translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 423-847 of SEQ ID NO: 5; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 423-847 of SEQ ID NO: 5; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 423-847 of SEQ ID NO: 5; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 423-847 of SEQ ID NO: 5; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 423-847 of SEQ ID NO: 5; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 423-847 of SEQ ID NO: 5.

In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/F translocation domain. In an aspect of this embodiment, a BoNT/F translocation domain comprises amino acids 440-866 of SEQ ID NO: 6. In another aspect of this embodiment, a BoNT/F translocation domain comprises a naturally occurring BoNT/F translocation domain variant, such as, e.g., a translocation domain from a BoNT/F isoform or a translocation domain from a BoNT/F subtype. In another aspect of this embodiment, a BoNT/F translocation domain comprises amino acids 440-866 of a naturally occurring BoNT/F translocation domain variant of SEQ ID NO: 6, such as, e.g., amino acids 440-866 of a BoNT/F isoform of SEQ ID NO: 6 or amino acids 440-866 of a BoNT/F subtype of SEQ ID NO: 6. In still another aspect of this embodiment, a BoNT/F translocation domain comprises a non-naturally occurring BoNT/F translocation domain variant, such as, e.g., a conservative BoNT/F translocation domain variant, a non-conservative BoNT/F translocation domain variant, a BoNT/F chimeric translocation domain, an active BoNT/F translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/F translocation domain comprises amino acids 440-866 of a non-naturally occurring BoNT/F translocation domain variant of SEQ ID NO: 6, such as, e.g., amino acids 440-866 of a conservative BoNT/F translocation domain variant of SEQ ID NO: 6, amino acids 440-866 of a non-conservative BoNT/F translocation domain variant of SEQ ID NO: 6, amino acids 440-866 of an active BoNT/F translocation domain fragment of SEQ ID NO: 6, or any combination thereof.

In other aspects of this embodiment, a BoNT/F translocation domain comprises a polypeptide having an amino acid identity to amino acids 440-866 of SEQ ID NO: 6 of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%. In yet other aspects of this embodiment, a BoNT/F translocation domain comprises a polypeptide having an amino acid identity to amino acids 440-866 of SEQ ID NO: 6 of, e.g., at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95%.

In other aspects of this embodiment, a BoNT/F translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 440-866 of SEQ ID NO: 6; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 440-866 of SEQ ID NO: 6; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 440-866 of SEQ ID NO: 6; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 440-866 of SEQ ID NO: 6; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 440-866 of SEQ ID NO: 6; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 440-866 of SEQ ID NO: 6.

In other aspects of this embodiment, a BoNT/F translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 440-866 of SEQ ID NO: 6; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 440-866 of SEQ ID NO: 6; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 440-866 of SEQ ID NO: 6; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 440-866 of SEQ ID NO: 6; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 440-866 of SEQ ID NO: 6; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 440-866 of SEQ ID NO: 6.

In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/G translocation domain. In an aspect of this embodiment, a BoNT/G translocation domain comprises amino acids 447-865 of SEQ ID NO: 7. In another aspect of this embodiment, a BoNT/G translocation domain comprises a naturally occurring BoNT/G translocation domain variant, such as, e.g., a translocation domain from a BoNT/G isoform or a translocation domain from a BoNT/G subtype. In another aspect of this embodiment, a BoNT/G translocation domain comprises amino acids 447-865 of a naturally occurring BoNT/G translocation domain variant of SEQ ID NO: 7, such as, e.g., amino acids 447-865 of a BoNT/G isoform of SEQ ID NO: 7 or amino acids 447-865 of a BoNT/G subtype of SEQ ID NO: 7. In still another aspect of this embodiment, a BoNT/G translocation domain comprises a non-naturally occurring BoNT/G translocation domain variant, such as, e.g., a conservative BoNT/G translocation domain variant, a non-conservative BoNT/G translocation domain variant, a BoNT/G chimeric translocation domain, an active BoNT/G translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/G translocation domain comprises amino acids 447-865 of a non-naturally occurring BoNT/G translocation domain variant of SEQ ID NO: 7, such as, e.g., amino acids 447-865 of a conservative BoNT/G translocation domain variant of SEQ ID NO: 7, amino acids 447-865 of a non-conservative BoNT/G translocation domain variant of SEQ ID NO: 7, amino acids 447-865 of an active BoNT/G translocation domain fragment of SEQ ID NO: 7, or any combination thereof. In other aspects of this embodiment, a BoNT/G translocation domain comprises a polypeptide having an amino acid identity to amino acids 447-865 of SEQ ID NO: 7 of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%. In yet other aspects of this embodiment, a BoNT/G translocation domain comprises a polypeptide having an amino acid identity to amino acids 447-865 of SEQ ID NO: 7 of, e.g., at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95%.

In other aspects of this embodiment, a BoNT/G translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 447-865 of SEQ ID NO: 7; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 447-865 of SEQ ID NO: 7; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 447-865 of SEQ ID NO: 7; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 447-865 of SEQ ID NO: 7; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 447-865 of SEQ ID NO: 7; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 447-865 of SEQ ID NO: 7.

In other aspects of this embodiment, a BoNT/G translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 447-865 of SEQ ID NO: 7; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 447-865 of SEQ ID NO: 7; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 447-865 of SEQ ID NO: 7; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 447-865 of SEQ ID NO: 7; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 447-865 of SEQ ID NO: 7.

In another embodiment, a Clostridial toxin translocation domain comprises a TeNT translocation domain. In an aspect of this embodiment, a TeNT translocation domain comprises amino acids 458-881 of SEQ ID NO: 8. In another aspect of this embodiment, a TeNT translocation domain comprises a naturally occurring TeNT translocation domain variant, such as, e.g., a translocation domain from a TeNT isoform or a translocation domain from a TeNT subtype. In another aspect of this embodiment, a TeNT translocation domain comprises amino acids 458-881 of a naturally occurring TeNT translocation domain variant of SEQ ID NO: 8, such as, e.g., amino acids 458-881 of a TeNT isoform of SEQ ID NO: 8 or amino acids 458-881 of a TeNT subtype of SEQ ID NO: 8. In still another aspect of this embodiment, a TeNT translocation domain comprises a non-naturally occurring TeNT translocation domain variant, such as, e.g., a conservative TeNT translocation domain variant, a non-conservative TeNT translocation domain variant, a TeNT chimeric translocation domain, an active TeNT translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a TeNT translocation domain comprises amino acids 458-881 of a non-naturally occurring TeNT translocation domain variant of SEQ ID NO: 8, such as, e.g., amino acids 458-881 of a conservative TeNT translocation domain variant of SEQ ID NO: 8, amino acids 458-881 of a non-conservative TeNT translocation domain variant of SEQ ID NO: 8, amino acids 458-881 of an active TeNT translocation domain fragment of SEQ ID NO: 8, or any combination thereof.

In other aspects of this embodiment, a TeNT translocation domain comprises a polypeptide having an amino acid identity to amino acids 458-881 of SEQ ID NO: 8 of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%. In yet other aspects of this embodiment, a TeNT translocation domain comprises a polypeptide having an amino acid identity to amino acids 458-881 of SEQ ID NO: 8 of, e.g., at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95%.

In other aspects of this embodiment, a TeNT translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 458-881 of SEQ ID NO: 8; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 458-881 of SEQ ID NO: 8; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 458-881 of SEQ ID NO: 8;

at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 458-881 of SEQ ID NO: 8; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 458-881 of SEQ ID NO: 8; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 458-881 of SEQ ID NO: 8.

In other aspects of this embodiment, a TeNT translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 458-881 of SEQ ID NO: 8; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 458-881 of SEQ ID NO: 8; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 458-881 of SEQ ID NO: 8; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 458-881 of SEQ ID NO: 8; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 458-881 of SEQ ID NO: 8; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 458-881 of SEQ ID NO: 8.

In another embodiment, a Clostridial toxin translocation domain comprises a BaNT translocation domain. In an aspect of this embodiment, a BaNT translocation domain comprises amino acids 432-857 of SEQ ID NO: 9. In another aspect of this embodiment, a BaNT translocation domain comprises a naturally occurring BaNT translocation domain variant, such as, e.g., a translocation domain from a BaNT isoform or a translocation domain from a BaNT subtype. In another aspect of this embodiment, a BaNT translocation domain comprises amino acids 432-857 of a naturally occurring BaNT translocation domain variant of SEQ ID NO: 9, such as, e.g., amino acids 432-857 of a BaNT isoform of SEQ ID NO: 9 or amino acids 432-857 of a BaNT subtype of SEQ ID NO: 9. In still another aspect of this embodiment, a BaNT translocation domain comprises a non-naturally occurring BaNT translocation domain variant, such as, e.g., a conservative BaNT translocation domain variant, a non-conservative BaNT translocation domain variant, a BaNT chimeric translocation domain, an active BaNT translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BaNT translocation domain comprises amino acids 432-857 of a non-naturally occurring BaNT translocation domain variant of SEQ ID NO: 9, such as, e.g., amino acids 432-857 of a conservative BaNT translocation domain variant of SEQ ID NO: 9, amino acids 432-857 of a non-conservative BaNT translocation domain variant of SEQ ID NO: 9, amino acids 432-857 of an active BaNT translocation domain fragment of SEQ ID NO: 9, or any combination thereof.

In other aspects of this embodiment, a BaNT translocation domain comprises a polypeptide having an amino acid identity to amino acids 432-857 of SEQ ID NO: 9 of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%. In yet other aspects of this embodiment, a BaNT translocation domain comprises a polypeptide having an amino acid identity to amino acids 432-857 of SEQ ID NO: 9 of, e.g., at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95%.

In other aspects of this embodiment, a BaNT translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 432-857 of SEQ ID NO: 9; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 432-857 of SEQ ID NO: 9; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 432-857 of SEQ ID NO: 9; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 432-857 of SEQ ID NO: 9; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 432-857 of SEQ ID NO: 9; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 432-857 of SEQ ID NO: 9.

In other aspects of this embodiment, a BaNT translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 432-857 of SEQ ID NO: 9; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 432-857 of SEQ ID NO: 9; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 432-857 of SEQ ID NO: 9; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 432-857 of SEQ ID NO: 9; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 432-857 of SEQ ID NO: 9; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 432-857 of SEQ ID NO: 9.

In another embodiment, a Clostridial toxin translocation domain comprises a BuNT translocation domain. In an aspect of this embodiment, a BuNT translocation domain comprises amino acids 423-847 of SEQ ID NO: 10. In another aspect of this embodiment, a BuNT translocation domain comprises a naturally occurring BuNT translocation domain variant, such as, e.g., a translocation domain from a BuNT isoform or a translocation domain from a BuNT subtype. In another aspect of this embodiment, a BuNT translocation domain comprises amino acids 423-847 of a naturally occurring BuNT translocation domain variant of SEQ ID NO: 10, such as, e.g., amino acids 423-847 of a BuNT isoform of SEQ ID NO: 10 or amino acids 423-847 of a BuNT subtype of SEQ ID NO: 10. In still another aspect of this embodiment, a BuNT translocation domain comprises a non-naturally occurring BuNT translocation domain variant, such as, e.g., a conservative BuNT translocation domain variant, a non-conservative BuNT translocation domain variant, a BuNT chimeric translocation domain, an active BuNT translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BuNT translocation domain comprises amino acids 423-847 of a non-naturally occurring BuNT translocation domain variant of SEQ ID NO: 10, such as, e.g., amino acids 423-847 of a conservative BuNT translocation domain variant of SEQ ID NO: 10, amino acids 423-847 of a non-conservative BuNT translocation domain variant of SEQ ID NO: 10, amino acids 423-847 of an active BuNT translocation domain fragment of SEQ ID NO: 10, or any combination thereof.

In other aspects of this embodiment, a BuNT translocation domain comprises a polypeptide having an amino acid identity to amino acids 423-847 of SEQ ID NO: 10 of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%. In yet other aspects of this embodiment, a BuNT translocation domain comprises a polypeptide having an amino acid identity to amino acids 423-847 of SEQ ID NO: 10 of, e.g., at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95%.

In other aspects of this embodiment, a BuNT translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 423-847 of SEQ ID NO: 10; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid substitutions relative to amino acids 423-847 of SEQ ID NO: 10; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 423-847 of SEQ ID NO: 10; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions relative to amino acids 423-847 of SEQ ID NO: 10; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 423-847 of SEQ ID NO: 10; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid additions relative to amino acids 423-847 of SEQ ID NO: 10.

In other aspects of this embodiment, a BuNT translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 423-847 of SEQ ID NO: 10; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid substitutions relative to amino acids 423-847 of SEQ ID NO: 10; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 423-847 of SEQ ID NO: 10; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions relative to amino acids 423-847 of SEQ ID NO: 10; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 423-847 of SEQ ID NO: 10; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid additions relative to amino acids 423-847 of SEQ ID NO: 10.

In another aspect of the invention, a modified Clostridial toxin comprises, in part, an opioid peptide binding domain. By "binding domain" is meant an amino acid sequence region able to preferentially bind to a cell surface marker characteristic of the target cell under physiological conditions. The cell surface marker may comprise a polypeptide, a polysaccharide, a lipid, a glycoprotein, a lipoprotein, or may have structural characteristics of more than one of these. By "preferentially interact" is meant that the disassociation constant (K_d) of the binding domain for the cell surface marker is at least one order of magnitude less than that of the binding domain for any other cell surface marker. Preferably, the disassociation constant is at least 2 orders of magnitude less, even more preferably the disassociation constant is at least 3 orders of magnitude less than that of the binding domain for any other cell surface marker to which the neurotoxin or modified neurotoxin is exposed. Examples of binding domains are described in, e.g., Steward, L. E. et al., Modified Clostridial Toxins with Enhanced Translocation Capability and Enhanced Targeting Activity, U.S. patent application Ser. No. 11/776,043 (Jul. 11, 2007); Steward, L. E. et al., Modified Clostridial Toxins with Enhanced Translocation Capabilities and Altered Targeting Activity For Clostridial Toxin Target Cells, U.S. patent application Ser. No. 11/776,052 (Jul. 11, 2007); and Steward, L. E. et al., Modified Clostridial Toxins with Enhanced Translocation Capabilities and Altered Targeting Activity For Non-Clostridial Toxin Target Cells, U.S. patent application Ser. No. 11/776,075 (Jul. 11, 2007), each of which is incorporated by reference in its entirety.

A non-limiting example of an opioid peptide binding domain disclosed in the present specification is, e.g., an enkephalin, an endomorphin, an endorphin, a dynorphin, a nociceptin or a hemorphin. Thus, in an embodiment, a binding domain comprises an opioid peptide.

In another embodiment, an opioid peptide comprises an enkephalin peptide. In aspects of this embodiment, a enkephalin peptide comprises a Leu-enkephalin, a Met-enkephalin, a Met-enkephalin MRGL or a Met-enkephalin MRF. In other aspects of this embodiment, an enkephalin peptide comprises SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54 or SEQ ID NO: 55.

In other aspects of this embodiment, an enkephalin comprises a polypeptide having an amino acid identity to SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54 or SEQ ID NO: 55 of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%. In yet other aspects of this embodiment, an enkephalin comprises a polypeptide having an amino acid identity to SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54 or SEQ ID NO: 55 of, e.g., at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95%.

In other aspects of this embodiment, an enkephalin comprises a polypeptide having, e.g., at least 1, 2, or 3 non-contiguous amino acid substitutions relative to SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54 or SEQ ID NO: 55; at most 1, 2, or 3 non-contiguous amino acid substitutions relative to SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54 or SEQ ID NO: 55; at least 1, 2, or 3 non-contiguous amino acid deletions relative to SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54 or SEQ ID NO: 55; at most 1, 2, or 3 non-contiguous amino acid deletions relative to SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54 or SEQ ID NO: 55; at least 1, 2, or 3 non-contiguous amino acid additions relative to SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54 or SEQ ID NO: 55; or at most 1, 2, or 3 non-contiguous amino acid additions relative to SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54 or SEQ ID NO: 55.

In other aspects of this embodiment, an enkephalin comprises a polypeptide having, e.g., at least 1, 2, or 3 contiguous amino acid substitutions relative to SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54 or SEQ ID NO: 55; at most 1, 2, or 3 contiguous amino acid substitutions relative to SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54 or SEQ ID NO: 55; at least 1, 2, or 3 contiguous amino acid deletions relative to SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54 or SEQ ID NO: 55; at most 1, 2, or 3 contiguous amino acid deletions relative to SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54 or SEQ ID NO: 55; at least 1, 2, or 3 contiguous amino acid additions relative to SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54 or SEQ ID NO: 55; or at most 1, 2, or 3 contiguous amino acid additions relative to SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54 or SEQ ID NO: 55.

In another embodiment, an opioid peptide comprises a bovine adrenomedullary-22 (BAM22) peptide. In aspects of this embodiment, a BAM22 peptide comprises a BAM22 peptide (1-12), a BAM22 peptide (6-22), a BAM22 peptide (8-22) or a BAM22 peptide (1-22). In other aspects of this embodiment, a BAM22 peptide comprises amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 56; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 57; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 58; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 59; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 60 or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 61.

In other aspects of this embodiment, a BAM22 comprises a polypeptide having an amino acid identity to amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22

of SEQ ID NO: 56; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 57; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 58; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 59; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 60; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 61 of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%. In yet other aspects of this embodiment, a BAM22 peptide binding domain comprises a polypeptide having an amino acid identity to amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 56; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 57; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 58; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 59; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 60; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 61 of, e.g., at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95%.

In other aspects of this embodiment, a BAM22 peptide comprises a polypeptide having, e.g., at least 1, 2, 3, 4, or 5 non-contiguous amino acid substitutions relative to amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 56; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 57; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 58; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 59; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 60; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 61; at most 1, 2, 3, 4, or 5 non-contiguous amino acid substitutions relative to amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 56; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 57; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 58; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 59; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 60; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 61; at least 1, 2, 3, 4, or 5 non-contiguous amino acid deletions relative to amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 56; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 57; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 58; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 59; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 60; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 61; at least 1, 2, 3, 4, or 5 non-contiguous amino acid additions relative to amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 56; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 57; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 58; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 59; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 60; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 61; at most 1, 2, 3, 4, or 5 non-contiguous amino acid additions relative to amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 56; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 57; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 58; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 59; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 60; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 61.

In other aspects of this embodiment, a BAM22 comprises a polypeptide having, e.g., at least 1, 2, 3, 4, or 5 contiguous amino acid substitutions relative to amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 56; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 57; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 58; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 59; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 60; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 61; at most 1, 2, 3, 4, or 5 contiguous amino acid substitutions relative to amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 56; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 57; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 58; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 59; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 60; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 61; at least 1, 2, 3, 4, or 5 contiguous amino acid deletions relative to amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 56; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 57; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 58; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 59; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 60; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 61; at most 1, 2, 3, 4, or 5 contiguous amino acid deletions relative to amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 56; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 57; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 58; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 59; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 60; or amino acids 1-12,

amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 61; at least 1, 2, 3, 4, or 5 contiguous amino acid additions relative to amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 56; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 57; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 58; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 59; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 60; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 61; or at most 1, 2, 3, 4, or 5 contiguous amino acid additions relative to amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 56; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 57; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 58; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 59; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 60; or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 61.

In another embodiment, an opioid peptide comprises an endomorphin peptide. In aspects of this embodiment, an endomorphin peptide comprises an endomorphin-1 or an endomorphin-2. In other aspects of this embodiment, an endomorphin peptide comprises SEQ ID NO: 62 or SEQ ID NO: 63.

In other aspects of this embodiment, an endomorphin comprises a polypeptide having an amino acid identity to SEQ ID NO: 62 or SEQ ID NO: 63 of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%. In yet other aspects of this embodiment, an endomorphin comprises a polypeptide having an amino acid identity to SEQ ID NO: 62 or SEQ ID NO: 63 of, e.g., at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95%.

In other aspects of this embodiment, an endomorphin comprises a polypeptide having, e.g., at least 1, 2, or 3 non-contiguous amino acid substitutions relative to SEQ ID NO: 62 or SEQ ID NO: 63; at most 1, 2, or 3 non-contiguous amino acid substitutions relative to SEQ ID NO: 62 or SEQ ID NO: 63; at least 1, 2, or 3 non-contiguous amino acid deletions relative to SEQ ID NO: 62 or SEQ ID NO: 63; at most 1, 2, or 3 non-contiguous amino acid deletions relative to SEQ ID NO: 62 or SEQ ID NO: 63; at least 1, 2, or 3 non-contiguous amino acid additions relative to SEQ ID NO: 62 or SEQ ID NO: 63; or at most 1, 2, or 3 non-contiguous amino acid additions relative to SEQ ID NO: 62 or SEQ ID NO: 63.

In other aspects of this embodiment, an endomorphin comprises a polypeptide having, e.g., at least 1, 2, or 3 contiguous amino acid substitutions relative to SEQ ID NO: 62 or SEQ ID NO: 63; at most 1, 2, or 3 contiguous amino acid substitutions relative to SEQ ID NO: 62 or SEQ ID NO: 63; at least 1, 2, or 3 contiguous amino acid deletions relative to SEQ ID NO: 62 or SEQ ID NO: 63; at most 1, 2, or 3 contiguous amino acid deletions relative to SEQ ID NO: 62 or SEQ ID NO: 63; at least 1, 2, or 3 contiguous amino acid additions relative to SEQ ID NO: 62 or SEQ ID NO: 63; or at most 1, 2, or 3 contiguous amino acid additions relative to SEQ ID NO: 62 or SEQ ID NO: 63.

In another embodiment, an opioid peptide comprises an endorphin peptide. In aspects of this embodiment, an endorphin peptide comprises an endorphin-α, a neoendorphin-α, an endorphin-β, a neoendorphin-β or an endorphin-γ. In other aspects of this embodiment, an endorphin peptide comprises SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68 or SEQ ID NO: 69.

In other aspects of this embodiment, an endorphin comprises a polypeptide having an amino acid identity to SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68 or SEQ ID NO: 69 of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%. In yet other aspects of this embodiment, an endorphin comprises a polypeptide having an amino acid identity to SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68 or SEQ ID NO: 69 of, e.g., at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95%.

In other aspects of this embodiment, an endorphin comprises a polypeptide having, e.g., at least 1, 2, 3, 4, or 5 non-contiguous amino acid substitutions relative to SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68 or SEQ ID NO: 69; at most 1, 2, 3, 4, or 5 non-contiguous amino acid substitutions relative to SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68 or SEQ ID NO: 69; at least 1, 2, 3, 4, or 5 non-contiguous amino acid deletions relative to SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68 or SEQ ID NO: 69; at most 1, 2, 3, 4, or 5 non-contiguous amino acid deletions relative to SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68 or SEQ ID NO: 69; at least 1, 2, 3, 4, or 5 non-contiguous amino acid additions relative to SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68 or SEQ ID NO: 69; or at most 1, 2, 3, 4, or 5 non-contiguous amino acid additions relative to SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68 or SEQ ID NO: 69.

In other aspects of this embodiment, an endorphin comprises a polypeptide having, e.g., at least 1, 2, 3, 4, or 5 contiguous amino acid substitutions relative to SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68 or SEQ ID NO: 69; at most 1, 2, 3, 4, or 5 contiguous amino acid substitutions relative to SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68 or SEQ ID NO: 69; at least 1, 2, 3, 4, or 5 contiguous amino acid deletions relative to SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68 or SEQ ID NO: 69; at most 1, 2, 3, 4, or 5 contiguous amino acid deletions relative to SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68 or SEQ ID NO: 69; at least 1, 2, 3, 4, or 5 contiguous amino acid additions relative to SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68 or SEQ ID NO: 69; or at most 1, 2, 3, 4, or 5 contiguous amino acid additions relative to SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68 or SEQ ID NO: 69.

In another embodiment, an opioid peptide comprises a dynorphin peptide. In aspects of this embodiment, a dynorphin peptide comprises a dynorphin A, a dynorphin B (leumorphin) or a rimorphin. In other aspects of this embodiment, a dynorphin peptide comprises SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99 or SEQ ID NO: 100.

In other aspects of this embodiment, a dynorphin comprises a polypeptide having an amino acid identity to SEQ ID NO: 70, SEQ ID NO: 79 or SEQ ID NO: 95 of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%. In yet other aspects of this embodiment, a dynorphin comprises a polypeptide having an amino acid identity to SEQ ID NO: 70, SEQ ID NO: 79 or SEQ ID NO: 95 of, e.g., at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95%.

In other aspects of this embodiment, a dynorphin comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid substitutions relative to SEQ ID NO: 70, SEQ ID NO: 79 or SEQ ID NO: 95; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid substitutions relative to SEQ ID NO: 70, SEQ ID NO: 79 or SEQ ID NO: 95; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid deletions relative to SEQ ID NO: 70, SEQ ID NO: 79 or SEQ ID NO: 95; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid deletions relative to SEQ ID NO: 70, SEQ ID NO: 79 or SEQ ID NO: 95; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid additions relative to SEQ ID NO: 70, SEQ ID NO: 79 or SEQ ID NO: 95; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid additions relative to SEQ ID NO: 70, SEQ ID NO: 79 or SEQ ID NO: 95.

In other aspects of this embodiment, a dynorphin comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid substitutions relative to SEQ ID NO: 70, SEQ ID NO: 79 or SEQ ID NO: 95; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid substitutions relative to SEQ ID NO: 70, SEQ ID NO: 79 or SEQ ID NO: 95; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid deletions relative to SEQ ID NO: 70, SEQ ID NO: 79 or SEQ ID NO: 95; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid deletions relative to SEQ ID NO: 70, SEQ ID NO: 79 or SEQ ID NO: 95; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid additions relative to SEQ ID NO: 70, SEQ ID NO: 79 or SEQ ID NO: 95; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid additions relative to SEQ ID NO: 70, SEQ ID NO: 79 or SEQ ID NO: 95.

In another embodiment, an opioid peptide comprises a nociceptin peptide. In aspects of this embodiment, a nociceptin peptide comprises a nociceptin RK, a nociceptin, a neuropeptide 1, a neuropeptide 2 or a neuropeptide 3. In other aspects of this embodiment, a nociceptin peptide comprises SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109 or SEQ ID NO: 110.

In other aspects of this embodiment, a nociceptin comprises a polypeptide having an amino acid identity to SEQ ID NO: 101, SEQ ID NO: 108, SEQ ID NO: 109 or SEQ ID NO: 110 of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%. In yet other aspects of this embodiment, a nociceptin comprises a polypeptide having an amino acid identity to SEQ ID NO: 101, SEQ ID NO: 108, SEQ ID NO: 109 or SEQ ID NO: 110 of, e.g., at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95%.

In other aspects of this embodiment, a nociceptin comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid substitutions relative to SEQ ID NO: 101, SEQ ID NO: 108, SEQ ID NO: 109 or SEQ ID NO: 110; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid substitutions relative to SEQ ID NO: 101, SEQ ID NO: 108, SEQ ID NO: 109 or SEQ ID NO: 110; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid deletions relative to SEQ ID NO: 101, SEQ ID NO: 108, SEQ ID NO: 109 or SEQ ID NO: 110; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid deletions relative to SEQ ID NO: 101, SEQ ID NO: 108, SEQ ID NO: 109 or SEQ ID NO: 110; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid additions relative to SEQ ID NO: 101, SEQ ID NO: 108, SEQ ID NO: 109 or SEQ ID NO: 110; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid additions relative to SEQ ID NO: 101, SEQ ID NO: 108, SEQ ID NO: 109 or SEQ ID NO: 110.

In other aspects of this embodiment, a nociceptin comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid substitutions relative to SEQ ID NO: 101, SEQ ID NO: 108, SEQ ID NO: 109 or SEQ ID NO: 110; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid substitutions relative to SEQ ID NO: 101, SEQ ID NO: 108, SEQ ID NO: 109 or SEQ ID NO: 110; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid deletions relative to SEQ ID NO: 101, SEQ ID NO: 108, SEQ ID NO: 109 or SEQ ID NO: 110; at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid deletions relative to SEQ ID NO: 101, SEQ ID NO: 108, SEQ ID NO: 109 or SEQ ID NO: 110; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid additions relative to SEQ ID NO: 101, SEQ ID NO: 108, SEQ ID NO: 109 or SEQ ID NO: 110; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid additions relative to SEQ ID NO: 101, SEQ ID NO: 108, SEQ ID NO: 109 or SEQ ID NO: 110.

Clostridial toxins are each translated as a single-chain polypeptide of approximately 150 kDa that is subsequently cleaved by proteolytic scission within a disulfide loop by a naturally-occurring protease (FIG. **18**). This cleavage occurs within the discrete di-chain loop region created between two cysteine residues that form a disulfide bridge. This posttranslational processing yields a di-chain molecule comprising an approximately 50 kDa light chain (LC) and an approximately 100 kDa heavy chain (HC) held together by the single disulfide bond and non-covalent interactions between the two chains (FIG. **2**). To facilitate recombinant production of a modified Clostridial toxin, an exogenous protease cleavage site can be used to convert the single-chain polypeptide form of a modified Clostridial toxin disclosed in the present specification into the di-chain form. See, e.g., Steward, L. E. et al., Modified Clostridial Toxins with Enhanced Targeting Capabilities For Endogenous Clostridial Toxin Receptor Systems, U.S. Patent Publication No. US 2008/0096248 (Apr. 24, 2008); Steward, L. E. et al., Activatable Clostridial Toxins, U.S. Patent Publication No. US 2008/0032930 (Feb. 7, 2008); Steward, supra, (2007); Dolly, supra, (2007); Foster, supra, WO 2006/059093 (2006); and Foster, supra, WO 2006/059105 (2006), each of which is hereby incorporated by reference in its entirety.

In is envisioned that any and all protease cleavage sites can be used to convert the single-chain polypeptide form of a Clostridial toxin into the di-chain form, including, without limitation, endogenous di-chain loop protease cleavage sites and exogenous protease cleavage sites. Thus, in an aspect of the invention, a modified Clostridial toxin comprises, in part, an endogenous protease cleavage site within a di-chain loop region. In another aspect of the invention, a modified Clostridial toxin comprises, in part, an exogenous protease cleavage site within a di-chain loop region. As used herein, the term "di-chain loop region" means the amino acid sequence of a Clostridial toxin containing a protease cleavage site used to convert the single-chain form of a Clostridial toxin into the di-chain form. Non-limiting examples of a Clostridial toxin di-chain loop region, include, a di-chain loop region of BoNT/A comprising amino acids 430-454 of

SEQ ID NO: 1; a di-chain loop region of BoNT/B comprising amino acids 437-446 of SEQ ID NO: 2; a di-chain loop region of BoNT/C1 comprising amino acids 437-453 of SEQ ID NO: 3; a di-chain loop region of BoNT/D comprising amino acids 437-450 of SEQ ID NO: 4; a di-chain loop region of BoNT/E comprising amino acids 412-426 of SEQ ID NO: 5; a di-chain loop region of BoNT/F comprising amino acids 429-445 of SEQ ID NO: 6; a di-chain loop region of BoNT/G comprising amino acids 436-450 of SEQ ID NO: 7; and a di-chain loop region of TeNT comprising amino acids 439-467 of SEQ ID NO: 8 (Table 2).

TABLE 2

Di-chain Loop Region of Clostridial Toxins

Toxin	SEQ ID NO:	Light Chain Region	Di-chain Loop Region Containing the Naturally-occurring Protease Cleavage Site	Heavy Chain Region
BoNT/A	11	NMNFTKLKNFTGLFEFYKLL	CVRGIITSKTKSLDKGYNK*----ALNDLC	IKVNNWDL
BoNT/B	12	KQAYEEISKEHLAVYKIQM	CKSVK*------------------APGIC	IDVDNEDL
BoNT/C1	13	PALRKVNPENMLYLFTKF	CHKAIDGRSLYNK*-----------TLDC	RELLVKNTDL
BoNT/D	14	PALQKLSSESVVDLFTKV	CLRLTKNSR*--------------DDSTC	IKVKNNRL
BoNT/E	15	PRIITPITGRGLVKKIIRF	CKNIVSVKGIR*-------------KSIC	IEINNGEL
BoNT/F	16	PKIIDSIPDKGLVEKIVKF	CKSVIPRKGTK*-----------APPRLC	IRVNNSEL
BoNT/G	17	KEAYEEISLEHLVIYRIAM	CKPVMYKNTGK*-------------SEQC	IIVNNEDL
TeNT	18	TNAFRNVDGSGLVSKLIGL	CKKIIPPTNIRENLYNRTA*SLTDLGGELC	IKIKNEDL
BaNT	19	SRIVGPIPDNGLVERFVGL	CKS-IVSKKGTK*------------NSLC	IKVNNRDL
BuNT	20	PRIITPITGRGLVKKIIRF	CKN-IVSVKGIR*------------KSIC	IEINNGEL

The amino acid sequence displayed are as follows: BoNT/A, residues 410-462 of SEQ ID No: 1; BoNT/B, residues 418-454 of SEQ ID No: 2; BoNT/C1, residues 419-463 of SEQ ID No: 3; BoNT/D, residues 419-458 of SEQ ID No: 4; BoNT/E, residues 393-434 of SEQ ID No: 5; BoNT/F, residues 410-453 of SEQ ID No: 6; BoNT/G, residues 419-458 of SEQ ID No: 7; TeNT, residues 422-475 of SEQ ID No: 8; BaNT, residues 402-443 of SEQ ID No: 9; and BuNT, residues 393-434 of SEQ ID No: 10.
An asterisks (*) indicates the peptide bond that is cleaved by a Clostridial toxin protease.

As used herein, the term "endogenous di-chain loop protease cleavage site" is synonymous with a "naturally occurring di-chain loop protease cleavage site" and means a naturally occurring protease cleavage site found within the di-chain loop region of a naturally occurring Clostridial toxin and includes, without limitation, naturally occurring Clostridial toxin di-chain loop protease cleavage site variants, such as, e.g., Clostridial toxin di-chain loop protease cleavage site isoforms and Clostridial toxin di-chain loop protease cleavage site subtypes. Non-limiting examples of an endogenous protease cleavage site, include, e.g., a BoNT/A di-chain loop protease cleavage site, a BoNT/B di-chain loop protease cleavage site, a BoNT/C1 di-chain loop protease cleavage site, a BoNT/D di-chain loop protease cleavage site, a BoNT/E di-chain loop protease cleavage site, a BoNT/F di-chain loop protease cleavage site, a BoNT/G di-chain loop protease cleavage site and a TeNT di-chain loop protease cleavage site.

As mentioned above, Clostridial toxins are translated as a single-chain polypeptide of approximately 150 kDa that is subsequently cleaved by proteolytic scission within a disulfide loop by a naturally-occurring protease. This posttranslational processing yields a di-chain molecule comprising an approximately 50 kDa light chain (LC) and an approximately 100 kDa heavy chain (HC) held together by a single disulphide bond and noncovalent interactions. While the identity of the protease is currently unknown, the di-chain loop protease cleavage site for many Clostridial toxins has been determined. In BoNTs, cleavage at K448-A449 converts the single polypeptide form of BoNT/A into the di-chain form; cleavage at K441-A442 converts the single polypeptide form of BoNT/B into the di-chain form; cleavage at K449-T450 converts the single polypeptide form of BoNT/C1 into the di-chain form; cleavage at R445-D446 converts the single polypeptide form of BoNT/D into the di-chain form; cleavage at R422-K423 converts the single polypeptide form of BoNT/E into the di-chain form; cleavage at K439-A440 converts the single polypeptide form of BoNT/F into the di-chain form; and cleavage at K446-S447 converts the single polypeptide form of BoNT/G into the di-chain form. Proteolytic cleavage of the single polypeptide form of TeNT at A457-S458 results in the di-chain form. Proteolytic cleavage of the single polypeptide form of BaNT at K431-N432 results in the di-chain form. Proteolytic cleavage of the single polypeptide form of BuNT at R422-K423 results in the di-chain form. Such a di-chain loop protease cleavage site is operably-linked in-frame to a modified Clostridial toxin as a fusion protein. However, it should also be noted that additional cleavage sites within the di-chain loop also appear to be cleaved resulting in the generation of a small peptide fragment being lost. As a non-limiting example, BoNT/A single-chain polypeptide cleave ultimately results in the loss of a ten amino acid fragment within the di-chain loop.

Thus, in an embodiment, a protease cleavage site comprising an endogenous Clostridial toxin di-chain loop protease cleavage site is used to convert the single-chain toxin into the di-chain form. In aspects of this embodiment, conversion into the di-chain form by proteolytic cleavage occurs from a site comprising, e.g., a BoNT/A di-chain loop protease cleavage site, a BoNT/B di-chain loop protease cleavage site, a BoNT/C1 di-chain loop protease cleavage site, a BoNT/D di-chain loop protease cleavage site, a BoNT/E di-chain loop protease cleavage site, a BoNT/F di-chain loop protease cleavage site, a BoNT/G di-chain loop protease cleavage site, a TeNT di-

chain loop protease cleavage site, a BaNT di-chain loop protease cleavage site, or a BuNT di-chain loop protease cleavage site.

In other aspects of this embodiment, conversion into the di-chain form by proteolytic cleavage occurs from a site comprising, e.g., a di-chain loop region of BoNT/A comprising amino acids 430-454 of SEQ ID NO: 1; a di-chain loop region of BoNT/B comprising amino acids 437-446 of SEQ ID NO: 2; a di-chain loop region of BoNT/C1 comprising amino acids 437-453 of SEQ ID NO: 3; a di-chain loop region of BoNT/D comprising amino acids 437-450 of SEQ ID NO: 4; a di-chain loop region of BoNT/E comprising amino acids 412-426 of SEQ ID NO: 5; a di-chain loop region of BoNT/F comprising amino acids 429-445 of SEQ ID NO: 6; a di-chain loop region of BoNT/G comprising amino acids 436-450 of SEQ ID NO: 7; or a di-chain loop region of TeNT comprising amino acids 439-467 of SEQ ID NO: 8. a di-chain loop region of BaNT comprising amino acids 421-435 of SEQ ID NO: 9; or a di-chain loop region of BuNT comprising amino acids 412-426 of SEQ ID NO: 10.

It is also envisioned that an exogenous protease cleavage site can be used to convert the single-chain polypeptide form of a modified Clostridial toxin disclosed in the present specification into the di-chain form. As used herein, the term "exogenous protease cleavage site" is synonymous with a "non-naturally occurring protease cleavage site" or "non-native protease cleavage site" and means a protease cleavage site that is not normally present in a di-chain loop region from a naturally occurring Clostridial toxin, with the proviso that the exogenous protease cleavage site is not a human protease cleavage site or a protease cleavage site that is susceptible to a protease being expressed in the host cell that is expressing a construct encoding an activatable polypeptide disclosed in the present specification. It is envisioned that any and all exogenous protease cleavage sites can be used to convert the single-chain polypeptide form of a Clostridial toxin into the di-chain form are useful to practice aspects of the present invention. Non-limiting examples of exogenous protease cleavage sites include, e.g., a plant papain cleavage site, an insect papain cleavage site, a crustacian papain cleavage site, an enterokinase cleavage site, a human rhinovirus 3C protease cleavage site, a human enterovirus 3C protease cleavage site, a tobacco etch virus (TEV) protease cleavage site, a Tobacco Vein Mottling Virus (TVMV) cleavage site, a subtilisin cleavage site, a hydroxylamine cleavage site, or a Caspase 3 cleavage site.

It is envisioned that an exogenous protease cleavage site of any and all lengths can be useful in aspects of the present invention with the proviso that the exogenous protease cleavage site is capable of being cleaved by its respective protease. Thus, in aspects of this embodiment, an exogenous protease cleavage site can have a length of, e.g., at least 6 amino acids, at least 7 amino acids, at least 8 amino acids, at least 9 amino acids, at least 10 amino acids, at least 15 amino acids, at least 20 amino acids, at least 25 amino acids, at least 30 amino acids, at least 40 amino acids, at least 50 amino acids, or at least 60 amino acids. In other aspects of this embodiment, an exogenous protease cleavage site can have a length of, e.g., at most 6 amino acids, at most 7 amino acids, at most 8 amino acids, at most 9 amino acids, at most 10 amino acids, at most 15 amino acids, at most 20 amino acids, at most 25 amino acids, at most 30 amino acids, at most 40 amino acids, at most 50 amino acids, or at most 60 amino acids.

In an embodiment, an exogenous protease cleavage site is located within the di-chain loop of a modified Clostridial toxin. In aspects of this embodiment, a modified Clostridial toxin comprises an exogenous protease cleavage site comprises, e.g., a plant papain cleavage site, an insect papain cleavage site, a crustacian papain cleavage site, a non-human enterokinase protease cleavage site, a Tobacco Etch Virus protease cleavage site, a Tobacco Vein Mottling Virus protease cleavage site, a human rhinovirus 3C protease cleavage site, a human enterovirus 3C protease cleavage site, a subtilisin cleavage site, a hydroxylamine cleavage site, a SUMO/ULP-1 protease cleavage site, and a non-human Caspase 3 cleavage site. In other aspects of this embodiment, an exogenous protease cleavage site is located within the di-chain loop of, e.g., a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G, a modified TeNT, a modified BaNT, or a modified BuNT.

In an aspect of this embodiment, an exogenous protease cleavage site can comprise, e.g., a non-human enterokinase cleavage site is located within the di-chain loop of a modified Clostridial toxin. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a bovine enterokinase protease cleavage site located within the di-chain loop of a modified Clostridial toxin. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a bovine enterokinase protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises SEQ ID NO: 21. In still other aspects of this embodiment, a bovine enterokinase protease cleavage site is located within the di-chain loop of, e.g., a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G, a modified TeNT, a modified BaNT, or a modified BuNT.

In another aspect of this embodiment, an exogenous protease cleavage site can comprise, e.g., a Tobacco Etch Virus protease cleavage site is located within the di-chain loop of a modified Clostridial toxin. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a Tobacco Etch Virus protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises the consensus sequence E-P5-P4-Y-P2-Q*-G (SEQ ID NO: 22) or E-P5-P4-Y-P2-Q*-S (SEQ ID NO: 23), where P2, P4 and P5 can be any amino acid. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a Tobacco Etch Virus protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32 or SEQ ID NO: 33. In still other aspects of this embodiment, a Tobacco Etch Virus protease cleavage site is located within the di-chain loop of, e.g., a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G, a modified TeNT, a modified BaNT, or a modified BuNT.

In another aspect of this embodiment, an exogenous protease cleavage site can comprise, e.g., a Tobacco Vein Mottling Virus protease cleavage site is located within the di-chain loop of a modified Clostridial toxin. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a Tobacco Vein Mottling Virus protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises the consensus sequence P6-P5-V-R-F-Q*-G (SEQ ID NO: 113) or P6-P5-V-R-F-Q*-S (SEQ ID NO: 114), where P5 and P6 can be any amino acid. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a Tobacco Vein Mottling Virus protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises SEQ ID NO: 115, SEQ ID

NO: 116, SEQ ID NO: 117, or SEQ ID NO: 118. In still other aspects of this embodiment, a Tobacco Vein Mottling Virus protease cleavage site is located within the di-chain loop of, e.g., a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G, a modified TeNT, a modified BaNT, or a modified BuNT.

In still another aspect of this embodiment, an exogenous protease cleavage site can comprise, e.g., a human rhinovirus 3C protease cleavage site is located within the di-chain loop of a modified Clostridial toxin. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a human rhinovirus 3C protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises the consensus sequence P5-P4-L-F-Q*-G-P (SEQ ID NO: 34), where P4 is G, A, V, L, I, M, S or T and P5 can any amino acid, with D or E preferred. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a human rhinovirus 3C protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39 or SEQ ID NO: 40. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a human rhinovirus 3C protease located within the di-chain loop of a modified Clostridial toxin that can be cleaved by PRESCISSION®. In still other aspects of this embodiment, a human rhinovirus 3C protease cleavage site is located within the di-chain loop of, e.g., a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G, a modified TeNT, a modified BaNT, or a modified BuNT.

In yet another aspect of this embodiment, an exogenous protease cleavage site can comprise, e.g., a subtilisin cleavage site is located within the di-chain loop of a modified Clostridial toxin. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a subtilisin cleavage site located within the di-chain loop of a modified Clostridial toxin comprises the consensus sequence P6-P5-P4-P3-H*-Y (SEQ ID NO: 41) or P6-P5-P4-P3-Y-H* (SEQ ID NO: 42), where P3, P4 and P5 and P6 can be any amino acid. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a subtilisin cleavage site located within the di-chain loop of a modified Clostridial toxin comprises SEQ ID NO: 43, SEQ ID NO: 44, or SEQ ID NO: 45. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a subtilisin cleavage site located within the di-chain loop of a modified Clostridial toxin that can be cleaved by GENENASE®. In still other aspects of this embodiment, a subtilisin cleavage site is located within the di-chain loop of, e.g., a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G, a modified TeNT, a modified BaNT, or a modified BuNT.

In yet another aspect of this embodiment, an exogenous protease cleavage site can comprise, e.g., a hydroxylamine cleavage site is located within the di-chain loop of a modified Clostridial toxin. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a hydroxylamine cleavage site comprising multiples of the dipeptide N*G. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a hydroxylamine cleavage site located within the di-chain loop of a modified Clostridial toxin comprises SEQ ID NO: 46, or SEQ ID NO: 47. In still other aspects of this embodiment, a hydroxylamine cleavage site is located within the di-chain loop of, e.g., a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G, a modified TeNT, a modified BaNT, or a modified BuNT.

In yet another aspect of this embodiment, an exogenous protease cleavage site can comprise, e.g., a SUMO/ULP-1 protease cleavage site is located within the di-chain loop of a modified Clostridial toxin. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a SUMO/ULP-1 protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprising the consensus sequence G-G*-P1'-P2'-P3' (SEQ ID NO: 112), where P1', P2', and P3' can be any amino acid. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a SUMO/ULP-1 protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises SEQ ID NO: 48. In still other aspects of this embodiment, a SUMO/ULP-1 protease cleavage site is located within the di-chain loop of, e.g., a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G, a modified TeNT, a modified BaNT, or a modified BuNT.

In an aspect of this embodiment, an exogenous protease cleavage site can comprise, e.g., a non-human Caspase 3 cleavage site is located within the di-chain loop of a modified Clostridial toxin. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a mouse Caspase 3 protease cleavage site located within the di-chain loop of a modified Clostridial toxin. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a non-human Caspase 3 protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises the consensus sequence D-P3-P2-D*P1' (SEQ ID NO: 119), where P3 can be any amino acid, with E preferred, P2 can be any amino acid and P1' can any amino acid, with G or S preferred. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a non-human Caspase 3 protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprising SEQ ID NO: 120, SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, or SEQ ID NO: 125. In still other aspects of this embodiment, a bovine enterokinase protease cleavage site is located within the di-chain loop of, e.g., a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G, a modified TeNT, a modified BaNT, or a modified BuNT.

A di-chain loop region is modified to replace a naturally-occurring di-chain loop protease cleavage site for an exogenous protease cleavage site. In this modification, the naturally-occurring di-chain loop protease cleavage site is made inoperable and thus can not be cleaved by its protease. Only the exogenous protease cleavage site can be cleaved by its corresponding exogenous protease. In this type of modification, the exogenous protease site is operably-linked in-frame to a modified Clostridial toxin as a fusion protein and the site can be cleaved by its respective exogenous protease. Replacement of an endogenous di-chain loop protease cleavage site with an exogenous protease cleavage site can be a substitution of the sites where the exogenous site is engineered at the position approximating the cleavage site location of the endogenous site. Replacement of an endogenous di-chain loop protease cleavage site with an exogenous protease cleavage site can be an addition of an exogenous site where the exogenous site is engineered at the position different from the cleavage site location of the endogenous site, the endogenous

site being engineered to be inoperable. The location and kind of protease cleavage site may be critical because certain binding domains require a free amino-terminal or carboxyl-terminal amino acid. For example, when an opioid peptide binding domain is placed between two other domains, e.g., see FIG. **4**, a criterion for selection of a protease cleavage site could be whether the protease that cleaves its site leaves a flush cut, exposing the free amino-terminal or carboxyl-terminal of the binding domain necessary for selective binding of the binding domain to its receptor.

A naturally-occurring protease cleavage site can be made inoperable by altering at least the two amino acids flanking the peptide bond cleaved by the naturally-occurring di-chain loop protease. More extensive alterations can be made, with the proviso that the two cysteine residues of the di-chain loop region remain intact and the region can still form the disulfide bridge. Non-limiting examples of an amino acid alteration include deletion of an amino acid or replacement of the original amino acid with a different amino acid. Thus, in one embodiment, a naturally-occurring protease cleavage site is made inoperable by altering the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease. In other aspects of this embodiment, a naturally-occurring protease cleavage site is made inoperable by altering, e.g., at least three amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at least four amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at least five amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at least six amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at least seven amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at least eight amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at least nine amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at least ten amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at least 15 amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; or at least 20 amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease.

In still other aspects of this embodiment, a naturally-occurring di-chain protease cleavage site is made inoperable by altering, e.g., at most three amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at most four amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at most five amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at most six amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at most seven amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at most eight amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at most nine amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at most ten amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; at most 15 amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease; or at most 20 amino acids including the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease.

It is understood that a modified Clostridial toxin disclosed in the present specification can optionally further comprise a flexible region comprising a flexible spacer. A flexible region comprising flexible spacers can be used to adjust the length of a polypeptide region in order to optimize a characteristic, attribute or property of a polypeptide. As a non-limiting example, a polypeptide region comprising one or more flexible spacers in tandem can be use to better expose a protease cleavage site thereby facilitating cleavage of that site by a protease. As another non-limiting example, a polypeptide region comprising one or more flexible spacers in tandem can be use to better present an opioid peptide binding domain, thereby facilitating the binding of that binding domain to its receptor.

A flexible space comprising a peptide is at least one amino acid in length and comprises non-charged amino acids with small side-chain R groups, such as, e.g., glycine, alanine, valine, leucine or serine. Thus, in an embodiment a flexible spacer can have a length of, e.g., at least 1 amino acids, at least 2 amino acids, at least 3 amino acids, at least 4 amino acids, at least 5 amino acids, at least 6 amino acids, at least 7 amino acids, at least 8 amino acids, at least 9 amino acids, or at least 10 amino acids. In another embodiment, a flexible spacer can have a length of, e.g., at most 1 amino acids, at most 2 amino acids, at most 3 amino acids, at most 4 amino acids, at most 5 amino acids, at most 6 amino acids, at most 7 amino acids, at most 8 amino acids, at most 9 amino acids, or at most 10 amino acids. In still another embodiment, a flexible spacer can be, e.g., between 1-3 amino acids, between 2-4 amino acids, between 3-5 amino acids, between 4-6 amino acids, or between 5-7 amino acids. Non-limiting examples of a flexible spacer include, e.g., a G-spacers such as GGG, GGGG (SEQ ID NO: 49), and GGGGS (SEQ ID NO: 50) or an A-spacers such as AAA, AAAA (SEQ ID NO: 51) and AAAAV (SEQ ID NO: 111). Such a flexible region is operably-linked in-frame to the modified Clostridial toxin as a fusion protein.

Thus, in an embodiment, a modified Clostridial toxin disclosed in the present specification can further comprise a flexible region comprising a flexible spacer. In another embodiment, a modified Clostridial toxin disclosed in the present specification can further comprise flexible region comprising a plurality of flexible spacers in tandem. In aspects of this embodiment, a flexible region can comprise in tandem, e.g., at least 1 G-spacer, at least 2 G-spacers, at least 3 G-spacers, at least 4 G-spacers or at least 5 G-spacers. In other aspects of this embodiment, a flexible region can comprise in tandem, e.g., at most 1 G-spacer, at most 2 G-spacers, at most 3 G-spacers, at most 4 G-spacers or at most 5 G-spacers. In still other aspects of this embodiment, a flexible region can comprise in tandem, e.g., at least 1 A-spacer, at least 2 A-spacers, at least 3 A-spacers, at least 4 A-spacers or at least 5 A-spacers. In still other aspects of this embodiment, a flexible region can comprise in tandem, e.g., at most 1 A-spacer, at most 2 A-spacers, at most 3 A-spacers, at most 4 A-spacers or at most 5 A-spacers. In another aspect of this embodiment, a modified Clostridial toxin can comprise a flexible region comprising one or more copies of the same flexible spacers, one or more copies of different flexible-spacer regions, or any combination thereof.

In other aspects of this embodiment, a modified Clostridial toxin comprising a flexible spacer can be, e.g., a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modi-

fied BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G, a modified TeNT, a modified BaNT, or a modified BuNT.

It is envisioned that a modified Clostridial toxin disclosed in the present specification can comprise a flexible spacer in any and all locations with the proviso that modified Clostridial toxin is capable of performing the intoxication process. In aspects of this embodiment, a flexible spacer is positioned between, e.g., an enzymatic domain and a translocation domain, an enzymatic domain and an opioid peptide binding domain, an enzymatic domain and an exogenous protease cleavage site. In other aspects of this embodiment, a G-spacer is positioned between, e.g., an enzymatic domain and a translocation domain, an enzymatic domain and an opioid peptide binding domain, an enzymatic domain and an exogenous protease cleavage site. In other aspects of this embodiment, an A-spacer is positioned between, e.g., an enzymatic domain and a translocation domain, an enzymatic domain and an opioid peptide binding domain, an enzymatic domain and an exogenous protease cleavage site.

In other aspects of this embodiment, a flexible spacer is positioned between, e.g., an opioid peptide binding domain and a translocation domain, an opioid peptide binding domain and an enzymatic domain, an opioid peptide binding domain and an exogenous protease cleavage site. In other aspects of this embodiment, a G-spacer is positioned between, e.g., an opioid peptide binding domain and a translocation domain, an opioid peptide binding domain and an enzymatic domain, an opioid peptide binding domain and an exogenous protease cleavage site. In other aspects of this embodiment, an A-spacer is positioned between, e.g., an opioid peptide binding domain and a translocation domain, an opioid peptide binding domain and an enzymatic domain, an opioid peptide binding domain and an exogenous protease cleavage site.

In yet other aspects of this embodiment, a flexible spacer is positioned between, e.g., a translocation domain and an enzymatic domain, a translocation domain and an opioid peptide binding domain, a translocation domain and an exogenous protease cleavage site. In other aspects of this embodiment, a G-spacer is positioned between, e.g., a translocation domain and an enzymatic domain, a translocation domain and an opioid peptide binding domain, a translocation domain and an exogenous protease cleavage site. In other aspects of this embodiment, an A-spacer is positioned between, e.g., a translocation domain and an enzymatic domain, a translocation domain and an opioid peptide binding domain, a translocation domain and an exogenous protease cleavage site.

It is envisioned that a modified Clostridial toxin disclosed in the present specification can comprise an opioid peptide binding domain in any and all locations with the proviso that modified Clostridial toxin is capable of performing the intoxication process. Non-limiting examples include, locating an opioid peptide binding domain at the amino terminus of a modified Clostridial toxin; locating an opioid peptide binding domain between a Clostridial toxin enzymatic domain and a translocation domain of a modified Clostridial toxin; and locating an opioid peptide binding domain at the carboxyl terminus of a modified Clostridial toxin. Other non-limiting examples include, locating an opioid peptide binding domain between a Clostridial toxin enzymatic domain and a Clostridial toxin translocation domain of a modified Clostridial toxin. The enzymatic domain of naturally-occurring Clostridial toxins contains the native start methionine. Thus, in domain organizations where the enzymatic domain is not in the amino-terminal location an amino acid sequence comprising the start methionine should be placed in front of the amino-terminal domain. Likewise, where an opioid peptide binding domain is in the amino-terminal position, an amino acid sequence comprising a start methionine and a protease cleavage site may be operably-linked in situations in which an opioid peptide binding domain requires a free amino terminus, see, e.g., Shengwen Li et al., Degradable Clostridial Toxins, U.S. patent application Ser. No. 11/572,512 (Jan. 23, 2007), which is hereby incorporated by reference in its entirety. In addition, it is known in the art that when adding a polypeptide that is operably-linked to the amino terminus of another polypeptide comprising the start methionine that the original methionine residue can be deleted.

Thus, in an embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising an opioid peptide binding domain, a translocation domain, an exogenous protease cleavage site and an enzymatic domain (FIG. **3**A). In an aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising an opioid peptide binding domain, a Clostridial toxin translocation domain, an exogenous protease cleavage site and a Clostridial toxin enzymatic domain.

In another embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising an opioid peptide binding domain, an enzymatic domain, an exogenous protease cleavage site, and a translocation domain (FIG. **3**B). In an aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising an opioid peptide binding domain, a Clostridial toxin enzymatic domain, an exogenous protease cleavage site, a Clostridial toxin translocation domain.

In yet another embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising an enzymatic domain, an exogenous protease cleavage site, an opioid peptide binding domain, and a translocation domain (FIG. **4**A). In an aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin enzymatic domain, an exogenous protease cleavage site, an opioid peptide binding domain, and a Clostridial toxin translocation domain.

In yet another embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a translocation domain, an exogenous protease cleavage site, an opioid peptide binding domain, and an enzymatic domain (FIG. **4**B). In an aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin translocation domain, an opioid peptide binding domain, an exogenous protease cleavage site and a Clostridial toxin enzymatic domain.

In another embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising an enzymatic domain, an opioid peptide binding domain, an exogenous protease cleavage site, and a translocation domain (FIG. **4**C). In an aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin enzymatic domain, an opioid peptide binding domain, an exogenous protease cleavage site, a Clostridial toxin translocation domain.

In yet another embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a translocation domain, an opioid peptide binding domain, an exogenous protease cleavage site and an enzymatic domain (FIG. **4**D). In an aspect of this embodi-

ment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin translocation domain, an opioid peptide binding domain, an exogenous protease cleavage site and a Clostridial toxin enzymatic domain.

In still another embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising an enzymatic domain, an exogenous protease cleavage site, a translocation domain, and an opioid peptide binding domain (FIG. **5**A). In an aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin enzymatic domain, an exogenous protease cleavage site, a Clostridial toxin translocation domain, and an opioid peptide binding domain.

In still another embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a translocation domain, an exogenous protease cleavage site, an enzymatic domain and an opioid peptide binding domain, (FIG. **5**B). In an aspect of this embodiment, a modified Clostridial toxin can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin translocation domain, an opioid peptide binding domain, an exogenous protease cleavage site and a Clostridial toxin enzymatic domain.

A composition useful in the invention generally is administered as a pharmaceutical acceptable composition comprising a modified Clostridial toxin. As used herein, the term "pharmaceutically acceptable" means any molecular entity or composition that does not produce an adverse, allergic or other untoward or unwanted reaction when administered to an individual. As used herein, the term "pharmaceutically acceptable composition" is synonymous with "pharmaceutical composition" and means a therapeutically effective concentration of an active ingredient, such as, e.g., any of the modified Clostridial toxins disclosed in the present specification. A pharmaceutical composition comprising a modified Clostridial toxin is useful for medical and veterinary applications. A pharmaceutical composition may be administered to a patient alone, or in combination with other supplementary active ingredients, agents, drugs or hormones. The pharmaceutical compositions may be manufactured using any of a variety of processes, including, without limitation, conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, and lyophilizing. The pharmaceutical composition can take any of a variety of forms including, without limitation, a sterile solution, suspension, emulsion, lyophilizate, tablet, pill, pellet, capsule, powder, syrup, elixir or any other dosage form suitable for administration.

Aspects of the present invention provide, in part, a composition comprising a modified Clostridial toxin. It is envisioned that any of the composition disclosed in the present specification can be useful in a method of treating urogenital-neurological disorder in a mammal in need thereof, with the proviso that the composition prevents or reduces a symptom associated with the urogenital-neurological disorder. Non-limiting examples of compositions comprising a modified Clostridial toxin include a modified Clostridial toxin comprising an opioid peptide binding domain, a Clostridial toxin translocation domain and a Clostridial toxin enzymatic domain. It is envisioned that any modified Clostridial toxin disclosed in the present specification can be used, including those disclosed in, e.g., Steward, supra, (2007); Dolly, supra, (2007); Foster, supra, WO 2006/059093 (2006); Foster, supra, WO 2006/059105 (Jun. 8, 2006). It is also understood that the two or more different modified Clostridial toxins can be provided as separate compositions or as part of a single composition.

It is also envisioned that a pharmaceutical composition comprising a modified Clostridial toxin can optionally include a pharmaceutically acceptable carriers that facilitate processing of an active ingredient into pharmaceutically acceptable compositions. As used herein, the term "pharmacologically acceptable carrier" is synonymous with "pharmacological carrier" and means any carrier that has substantially no long term or permanent detrimental effect when administered and encompasses terms such as "pharmacologically acceptable vehicle, stabilizer, diluent, additive, auxiliary or excipient." Such a carrier generally is mixed with an active compound, or permitted to dilute or enclose the active compound and can be a solid, semi-solid, or liquid agent. It is understood that the active ingredients can be soluble or can be delivered as a suspension in the desired carrier or diluent. Any of a variety of pharmaceutically acceptable carriers can be used including, without limitation, aqueous media such as, e.g., water, saline, glycine, hyaluronic acid and the like; solid carriers such as, e.g., mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like; solvents; dispersion media; coatings; antibacterial and antifungal agents; isotonic and absorption delaying agents; or any other inactive ingredient. Selection of a pharmacologically acceptable carrier can depend on the mode of administration. Except insofar as any pharmacologically acceptable carrier is incompatible with the active ingredient, its use in pharmaceutically acceptable compositions is contemplated. Non-limiting examples of specific uses of such pharmaceutical carriers can be found in Pharmaceutical Dosage Forms and Drug Delivery Systems (Howard C. Ansel et al., eds., Lippincott Williams & Wilkins Publishers, 7th ed. 1999); Remington: The Science and Practice of Pharmacy (Alfonso R. Gennaro ed., Lippincott, Williams & Wilkins, 20thed. 2000); Goodman & Gilman's The Pharmacological Basis of Therapeutics (Joel G. Hardman et al., eds., McGraw-Hill Professional, 10th ed. 2001); and Handbook of Pharmaceutical Excipients (Raymond C. Rowe et al., APhA Publications, 4th edition 2003). These protocols are routine procedures and any modifications are well within the scope of one skilled in the art and from the teaching herein.

It is further envisioned that a pharmaceutical composition disclosed in the present specification can optionally include, without limitation, other pharmaceutically acceptable components (or pharmaceutical components), including, without limitation, buffers, preservatives, tonicity adjusters, salts, antioxidants, osmolality adjusting agents, physiological substances, pharmacological substances, bulking agents, emulsifying agents, wetting agents, sweetening or flavoring agents, and the like. Various buffers and means for adjusting pH can be used to prepare a pharmaceutical composition disclosed in the present specification, provided that the resulting preparation is pharmaceutically acceptable. Such buffers include, without limitation, acetate buffers, citrate buffers, phosphate buffers, neutral buffered saline, phosphate buffered saline and borate buffers. It is understood that acids or bases can be used to adjust the pH of a composition as needed. Pharmaceutically acceptable antioxidants include, without limitation, sodium metabisulfite, sodium thiosulfate, acetylcysteine, butylated hydroxyanisole and butylated hydroxytoluene. Useful preservatives include, without limitation, benzalkonium chloride, chlorobutanol, thimerosal, phenylmercuric acetate, phenylmercuric nitrate, a stabilized oxy chloro composition, such as, e.g., PURITE® and chelants,

such as, e.g., DTPA or DTPA-bisamide, calcium DTPA, and CaNaDTPA-bisamide. Tonicity adjustors useful in a pharmaceutical composition include, without limitation, salts such as, e.g., sodium chloride, potassium chloride, mannitol or glycerin and other pharmaceutically acceptable tonicity adjustor. The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. It is understood that these and other substances known in the art of pharmacology can be included in a pharmaceutical composition useful in the invention.

In an embodiment, a composition comprising a modified Clostridial toxin is a pharmaceutical composition comprising a modified Clostridial toxin. In aspects of this embodiment, a pharmaceutical composition comprising a modified Clostridial toxin further comprises a pharmacological carrier, a pharmaceutical component, or both a pharmacological carrier and a pharmaceutical component. In other aspects of this embodiment, a pharmaceutical composition comprising a modified Clostridial toxin further comprises at least one pharmacological carrier, at least one pharmaceutical component, or at least one pharmacological carrier and at least one pharmaceutical component.

Aspects of the present invention provide, in part, an urogenital-neurological disorder. As used herein, the term "urogenital-neurological disorder" means an urogenital-rooted disorder where at least one of the underlying symptoms being treated is due to a nociceptive sensory nerve-based etiology, such as, e.g., a spastic dysfunction and/or degeneration of the sacral reflex arcs. Non-limiting examples of urogenital-neurological disorders, include, without limitation, urinary incontinence, overactive bladder, detrusor dysfunction, lower urinary tract dysfunction, urinary retention, urinary hesitancy, polyuria, nocturia, chronic urinary tract infection, prostate disorders associated with or without other urogenital disorders, uterine disorders associated with or without other urogenital disorders, and urogenital disorders associated with neurogenic dysfunction (such as, e.g., urogenital disorders associated with Parkinson's Disease, multiple sclerosis, spina bifida, transverse myelitis, stroke, spinal cord injury, spasm reflex, and a neurologic lesion of the spinal cord or brain), and other such urogenital disorders of a nociceptive sensory nerve-based etiology.

An individual's ability to hold urine and maintain continence depends on normal function of the lower urinary tract, the kidneys, and the nervous system. The individual must also have a physical and psychological ability to recognize and appropriately respond to the urge to urinate. The bladder's ability to fill and store urine requires a functional sphincter muscle (which controls the flow of urine out of the body) and a stable bladder wall muscle (detrusor). Normal bladder function is dependent on the nerves that sense the fullness of the bladder and on those that trigger the muscle movements that either empty it or retain urine. The process of urination involves two phases: 1) filling and storage of bladder and 2) emptying of bladder. During the filling and storage phase, the bladder stretches so it can hold the increasing amount of urine. The bladder of an average person can hold 350 mL to 550 mL of urine. Generally, the reflex to urinate is triggered when the bladder of an individual when approximately 200 mL of urine collects in the bladder. The emptying phase requires that the detrusor muscle contract, forcing urine out of the bladder through the urethra. The sphincter muscle must relax at the same time, so that urine can flow out of the body. The bladder, internal sphincters, and external sphincters may all be affected by nociceptive sensory nerve-based disorders that create abnormalities in bladder function. The damage can cause the bladder to be underactive, in which it is unable to contract and unable to empty completely, or it can be overactive, in which it contracts too quickly or frequently.

One type of urogenital-neurological disorder is urinary incontinence. Urinary incontinence is the inability to control the passage of urine. This can range from an occasional leakage of urine, to a complete inability to hold any urine. Urinary incontinence can be caused by abnormalities in bladder capacity or malfunction of control mechanisms such as the bladder neck and/or external urethral sphincter muscle that are important for the bladder's storage function. The many types of urinary incontinence.

Stress incontinence is a type of urinary incontinence in which the strength of the muscles (urethral sphincter) that help control urination is reduced as a result of weakened pelvic muscles that support the bladder and urethra or because of malfunction of the urethral sphincter. The weakness may be caused by prior injury to the urethral area, neurological injury, some medications, or after surgery of the prostate or pelvic area. The sphincter is not able to prevent urine flow when there is increased pressure from the abdomen such as during certain activities like coughing, sneezing, laughing, or exercise. Stress urinary incontinence is the most common type of urinary incontinence in women. Studies have shown about 50% of all women have occasional urinary incontinence, and as many as 10% have frequent incontinence. Nearly 20% of women over age 75 experience daily urinary incontinence. Stress incontinence is often seen in women who have had multiple pregnancies and vaginal childbirths, whose bladder, urethra, or rectal wall stick out into the vaginal space (pelvic prolapse).

Urge incontinence is a type of urinary incontinence that involves a strong, sudden need to urinate, followed by instant bladder contraction and involuntary loss of urine which results in leakage. There is not enough time between when an individual suffering from urge incontinence recognizes the need to urinate and when urination actually occurs. Urge incontinence is leakage of urine due to bladder muscles that contract inappropriately. Often these contractions occur regardless of the amount of urine that is in the bladder. Urge incontinence may result from neurological injuries (such as spinal cord injury or stroke), neurological dysfunction (such as, e.g., Parkinson's Disease and multiple sclerosis), infection, bladder cancer, bladder stones, bladder inflammation, or bladder outlet obstruction. In men, urge incontinence may be due to neurological disease or bladder changes caused by benign prostatic hypertrophy (BPH) or bladder outlet obstruction from an enlarged prostate. The majority of cases of urge incontinence are idiopathic, which means a specific cause cannot be identified. Although urge incontinence may occur in anyone at any age, it is more common in women and the elderly. Urge incontinence is also known as irritable bladder, spasmodic bladder, and unstable bladder.

Overflow urinary incontinence happens when small amounts of urine leak from a bladder that is always full. In older men, this can occur when the flow of urine from the bladder is blocked, usually by an enlarged prostate. It can sometimes be prevented by medication when early symptoms of prostate enlargement, such as frequent urination, appear. Some people with diabetes also have overflow incontinence. Mixed urinary incontinence describes a disorder where an individual exhibits symptoms associated with both stress incontinence and urge incontinence. Continuous urinary incontinence is the complaint of continuous leakage.

Thus in embodiment, a mammal suffering from urinary incontinence is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the urinary incontinence. In an aspect of this embodiment, a mammal suffering from stress incontinence is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the stress incontinence. In another aspect of this embodiment, a mammal suffering from urge incontinence is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the urge incontinence. In still another aspect of this embodiment, a mammal suffering from overflow urinary incontinence is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the overflow urinary incontinence. In a further aspect of this embodiment, a mammal suffering from mixed urinary incontinence is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the mixed urinary incontinence. In a further aspect of this embodiment, a mammal suffering from continuous urinary incontinence is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the continuous urinary incontinence.

Another type of urogenital-neurological disorder is overactive bladder. Overactive bladder is increased urinary urgency, with or without urge urinary incontinence, usually with frequency and nocturia. The individual may report symptoms of urinary urgency (the sudden, intense desire to urinate immediately), urinary frequency (the need to urinate more times than is normal), enuresis (any involuntary loss of urine), polyuria, nocturia, and/or urinary incontinence. Thus, overactive bladder describes a bladder that contracts more often than it should, so that a person feels the need to urinate more frequently and/or urgently than necessary and is characterized by uncontrolled, frequent expulsion of urine from the bladder. An overactive bladder usually, but not always, causes urinary incontinence. Individuals with overactive bladder may go to the bathroom very often, e.g., every two hours during the day and night, and may even wet the bed. Often, a strong urge to void is experienced when only a small amount of urine is in the bladder. There may be reduced bladder capacity and incomplete emptying of urine. An overactive bladder can be caused by interruptions in the nerve pathways to the bladder occurring above the sacrum. For example, spastic bladder may be caused by an inability of the detrusor muscle of the bladder to inhibit emptying contractions until a reasonable amount of urine has accumulated. As such, overactive bladder is often associated with detrusor overactivity, a pattern of bladder muscle contraction observed during urodynamics. Overactive bladder can also be caused by urinary tract infection, outflow obstruction and stress incontinence. Sometimes no cause is found, and such idiopathic cases may be due to anxiety or aging. Symptoms include the need to urinate may times throughout the day and night, the sensation of having to urinate immediately, and/or the sudden leakage of urine from the bladder.

Diseases extrinsic to the bladder may also cause the symptoms of overactive bladder. In the male patient, the extrinsic disorder most often responsible for overactive bladder is bladder outlet obstruction (BOO). Disorders extrinsic to the bladder in the female patient include urethral diverticulum, retroverted uterus, pelvic prolapse (including cystocele), gravid uterus, and loss or reduction of estrogen. Disorders extrinsic to the bladder common to both men and woman include pelvic mass, physiologic nocturnal diuresis, and polyuria caused by factors such as excessive fluid intake, diuretic use, or diabetes. Neuromuscular disorders may also account for the overactive bladder. Neurogenic disorders resulting from nerve damage to sensory nerves can also cause overactive bladder, including, without limitation, Parkinson disease, multiple sclerosis, spina bifida, cervical stenosis, spinal cord injury, diabetic neuropathy, pelvic surgery, or invertebral disc herniation, hydrocephalus, stroke, spinal cord injuries and lesions of the spinal cord or brain. Bladder aging may also account for these symptoms. A patient history of pelvic trauma, pelvic radiation, or bladder, prostate, or urethral surgery should also be considered when seeking to determine the etiology of the overactive bladder.

Thus in embodiment, a mammal suffering from overactive bladder is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the overactive bladder. In an aspect of this embodiment, a mammal suffering from overactive bladder is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces incontinence. In an aspect of this embodiment, a mammal suffering from overactive bladder is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces urinary frequency. In another aspect of this embodiment, a mammal suffering from overactive bladder is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces urinary urgency. In another aspect of this embodiment, a mammal suffering from overactive bladder is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces enuresis. In another aspect of this embodiment, a mammal suffering from overactive bladder is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces polyuria. In yet another aspect of this embodiment, a mammal suffering from overactive bladder is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces nocturia. In yet another aspect of this embodiment, a mammal suffering from overactive bladder is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces urinary incontinence.

Another type of urogenital-neurological disorder is detrusor dysfunction, including, without limitation, detrusor overactivity, detrusor instability, and detrusor-sphincter dyssynergia. One kind of detrusor dysfunction is detrusor overactivity or involuntary detrusor contractions (previously termed detrusor hyperreflexia). Detrusor overactivity involves increased involuntary contractions of the detrusor muscle during the filling phase which may be spontaneous or provoked resulting in uninhibitable bladder contractions. The muscle contraction patterns of detrusor overactivity include, without limitation, phasic detrusor overactivity and terminal detrusor overactivity. Detrusor overactivity can be either idiopathic in nature or they can be caused by non-neurogenic or neurogenic conditions. Symptoms of detrusor overactivity include, without limitation, uninhibitable bladder contractions, urinary urgency, urinary frequency, enuresis, polyuria, nocturia, and/or urinary incontinence. Another kind of detru-

sor dysfunction is detrusor instability. Detrusor instability involves uncontrolled involuntary contractions of the detrusor muscle resulting in uninhibitable bladder contractions irrespective of bladder capacity. Symptoms of detrusor instability include, without limitation, uninhibitable bladder contractions, urinary urgency, urinary frequency, enuresis, polyuria, nocturia, and/or urinary incontinence. Another kind of detrusor dysfunction is detrusor-sphincter dyssynergia (DSD). Detrusor-sphincter dyssynergia occurs when the contraction of the detrusor musculature is not coordinated with the relaxation of the sphincter thereby preventing the urethra from relaxing completely during voiding. Symptoms of detrusor-sphincter dyssynergia include, without limitation, urine flow interruption, raised detrusor pressure and/or urinary retention. DSD can be caused as a consequence of a neurological condition such as spinal injury or multiple sclerosis.

Thus in embodiment, a mammal suffering from detrusor dysfunction is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the detrusor dysfunction. In an aspect of this embodiment, a mammal suffering from detrusor dysfunction is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces uninhibitable bladder contractions. In another aspect of this embodiment, a mammal suffering from detrusor dysfunction is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces urinary frequency. In another aspect of this embodiment, a mammal suffering from detrusor dysfunction is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces urinary urgency. In yet another aspect of this embodiment, a mammal suffering from detrusor dysfunction is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces enuresis. In yet another aspect of this embodiment, a mammal suffering from detrusor dysfunction is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces polyuria. In yet another aspect of this embodiment, a mammal suffering from detrusor dysfunction is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces nocturia. In yet another aspect of this embodiment, a mammal suffering from detrusor dysfunction is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces urinary incontinence. In still another aspect of this embodiment, a mammal suffering from detrusor dysfunction is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces urine flow interruption. In still another aspect of this embodiment, a mammal suffering from detrusor dysfunction is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces detrusor pressure. In still another aspect of this embodiment, a mammal suffering from detrusor dysfunction is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces urinary retention.

In another embodiment, a mammal suffering from detrusor overactivity is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the detrusor overactivity. In an aspect of this embodiment, a mammal suffering from detrusor overactivity is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces uninhibitable bladder contractions. In another aspect of this embodiment, a mammal suffering from detrusor overactivity is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces urinary frequency. In another aspect of this embodiment, a mammal suffering from detrusor overactivity is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces urinary urgency. In yet another aspect of this embodiment, a mammal suffering from detrusor overactivity is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces enuresis. In yet another aspect of this embodiment, a mammal suffering from detrusor overactivity is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces polyuria. In yet another aspect of this embodiment, a mammal suffering from detrusor overactivity is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces nocturia. In yet another aspect of this embodiment, a mammal suffering from detrusor overactivity is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces urinary incontinence.

In yet another embodiment, a mammal suffering from detrusor instability is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the detrusor instability. In an aspect of this embodiment, a mammal suffering from detrusor instability is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces uninhibitable bladder contractions. In another aspect of this embodiment, a mammal suffering from detrusor instability is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces urinary frequency. In another aspect of this embodiment, a mammal suffering from detrusor instability is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces urinary urgency. In yet another aspect of this embodiment, a mammal suffering from detrusor instability is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces enuresis. In yet another aspect of this embodiment, a mammal suffering from detrusor instability is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces polyuria. In yet another aspect of this embodiment, a mammal suffering from detrusor instability is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces nocturia. In yet another aspect of this embodiment, a mammal suffering from detrusor instability is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces urinary incontinence.

In still another embodiment, a mammal suffering from detrusor-sphincter dyssynergia is treated with a composition

comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the detrusor-sphincter dyssynergia. In an aspect of this embodiment, a mammal suffering from detrusor-sphincter dyssynergia is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces urine flow interruption. In another aspect of this embodiment, a mammal suffering from detrusor-sphincter dyssynergia is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces detrusor pressure. In yet another aspect of this embodiment, a mammal suffering from detrusor-sphincter dyssynergia is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces urinary retention.

Another type of urogenital-neurological disorder is a lower urinary tract dysfunction (LUTD). See e.g., Paul Abrams et al., *The Standardisation of Terminology of Lower Urinary Tract Function: Report from the Standardisation Subcommittee of the International Continence Society,* 21 Neurourol Urodyn. 167-178 (2002), which is hereby incorporated by reference in its entirety. Lower urinary tract dysfunctions manifest three general types of symptoms: storage, voiding, and post-micturition symptoms. Storage symptoms are experienced during the storage phase of the bladder and include, without limitation, urinary urgency, urinary frequency, enuresis, polyuria, nocturia increased bladder sensation, decreased bladder sensation, absent bladder sensation, non-specific bladder sensation, and/or urinary incontinence. Voiding symptoms are experienced during the voiding phase. Symptoms include, without limitation, reduced urine flow, splitting or spraying of urine, intermittent urine flow, urinary hesitancy, strained effort to void urine, and/or terminal dribble of urine. Post-micturition symptoms are experienced immediately after micturition and include, without limitation, sensation of incomplete emptying and/or post-micturition dribble.

Thus in embodiment, a mammal suffering from a lower urinary tract dysfunction is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the lower urinary tract dysfunction. In an aspect of this embodiment, a mammal suffering from a lower urinary tract dysfunction is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces storage symptoms. In aspects of this embodiment, the storage symptom reduced is urinary urgency, urinary frequency, enuresis, polyuria, nocturia increased bladder sensation, decreased bladder sensation, absent bladder sensation, non-specific bladder sensation, or urinary incontinence. In another aspect of this embodiment, a mammal suffering from a lower urinary tract dysfunction is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces voiding symptoms. In aspects of this embodiment, the voiding symptom reduced is reduced urine flow, splitting or spraying of urine, intermittent urine flow, urinary hesitancy, strained effort to void urine, or terminal dribble of urine. In yet another aspect of this embodiment, a mammal suffering from a lower urinary tract dysfunction is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces post-micturition symptoms. In aspects of this embodiment, the post-micturition symptom reduced is sensation of incomplete emptying or post-micturition dribble.

Another type of urogenital-neurological disorder is urinary retention. Urinary retention is the inability to pass urine from the bladder and may be either an acute or chronic condition. Normally, the reflex to urinate is triggered when the bladder fills to approximately 300-500 mL. The bladder is then emptied when the contraction of the bladder wall forces urine out through the urethra. The bladder, internal sphincters, and external sphincters may all be affected by disorders that create abnormalities in bladder function resulting in urinary retention. Urinary retention can result either from loss of bladder muscle contracting performance or loss of appropriate coordination between the bladder muscle and the urethral sphincter muscle. The inability to properly relax the urinary sphincter muscles causing difficulty in emptying the bladder, which can lead to urinary retention. Often, a strong urge to void is experienced when only a small amount of urine is in the bladder. In addition, there may be reduced bladder capacity and incomplete emptying of urine. Urinary retention may also be caused by difficulty in relaxing the urinary sphincter muscle because the sphincter may be spastic. Alternatively, the bladder neck may be hypertrophied. Other causes of urinary retention include interruptions in the nerve pathways to the bladder occurring above the sacrum. This nerve damage results in a loss of sensation and motor control and is often seen in stroke, Parkinson's disease, spina bifida, diabetes, pelvic surgery, or invertebral disc herniation, and most forms of spinal cord injuries. Sometimes no cause is found, and such idiopathic cases may be due to anxiety or aging. Urinary retention can also occur by a blockage to the flow of urine due to prostate enlargement or urinary tract stones. Another type of urinary retention disorder is stones, which block the urinary tract of an individual thereby causing stoppage of urine flow and/or infection. Either chronic or acute retention may lead to incontinence due to leakage of urine from an overfull bladder.

Thus in embodiment, a mammal suffering from urinary retention is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the urinary retention. In an aspect of this embodiment, a mammal suffering from urinary retention is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces urinary urgency. In another aspect of this embodiment, a mammal suffering from urinary retention is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces urinary frequency. In yet another aspect of this embodiment, a mammal suffering from urinary retention is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration increases bladder capacity. In still another aspect of this embodiment, a mammal suffering from urinary retention is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces urinary incontinence. In still another aspect of this embodiment, a mammal suffering from urinary retention is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration restores normal urine flow.

In another embodiment, a mammal suffering from acute urinary retention is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the acute urinary retention. In yet another embodiment, a mammal suffering from chronic urinary retention is

treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the chronic urinary retention.

Another type of urogenital-neurological disorder is urinary hesitancy. Urinary hesitancy is difficulty starting or maintaining a urinary stream. This problem affects people of all ages and occurs in both sexes, but it is most common in older men with enlarged prostate glands. Urinary hesitancy usually comes on gradually. It sometimes goes unnoticed until urinary retention (complete inability to urinate) produces distention and discomfort in the bladder. Almost all older men have some degree of difficulty in starting urination, dribbling, or decreased force of the urinary stream. Urinary hesitancy can be caused by benign prostatic hyperplasia (enlarged prostate), urinary tract infection, especially if chronic and recurrent, prostatitis (inflammation or infection of the prostate gland), drugs (some cold remedies, some nasal decongestants, tricyclic antidepressants, and anticholinergics which may be used for incontinence), shy or bashful bladder syndrome in younger people (unable to urinate when another person is in the room), and neurological disorders.

Thus in embodiment, a mammal suffering from urinary hesitancy is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the urinary hesitancy. In an aspect of this embodiment, a mammal suffering from urinary hesitancy is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces urinary urgency. In another aspect of this embodiment, a mammal suffering from urinary hesitancy is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces urinary frequency. In yet another aspect of this embodiment, a mammal suffering from urinary hesitancy is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration increases bladder capacity. In still another aspect of this embodiment, a mammal suffering from urinary hesitancy is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces urinary incontinence. In still another aspect of this embodiment, a mammal suffering from urinary hesitancy is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration restores normal urine flow.

Another type of urogenital-neurological disorder is polyuria. Polyuria is when a person releases abnormally excessive volume of urine each day. An excessive volume of urination for an adult would be at least 2.5 liters of urine per day. Polyuria is a fairly common symptom, which is often noticed when you have to get up to use the bathroom at night. Thus in embodiment, a mammal suffering from polyuria is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the polyuria.

Another type of urogenital-neurological disorder is nocturia. Nocturia is excessive urination at night, such as by waking up several times during the night to urinate. Normally, urine decreases in amount and become more concentrated at night. That means, most people can sleep 6 to 8 hours without having to urinate. But, persons with nocturia get up more than once during the night to urinate. Because of this, those who have excessive urination at night often have disrupted sleep cycles. Causes include benign prostatic hyperplasia, certain drugs including diuretics, cardiac glycosides, demeclocycline, lithium, methoxyflurane, phenytoin, propoxyphene, and excessive vitamin D, chronic or recurrent urinary tract infection, chronic renal failure, congestive heart failure, cystitis, diabetes, drinking too much fluid before bedtime, particularly coffee, caffeinated beverages, or alcohol, and obstructive sleep apnea and other sleeping disorders. Thus in embodiment, a mammal suffering from nocturia is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the nocturia.

Another type of urogenital-neurological disorder is chronic urinary tract infection (recurrent infection). Chronic urinary tract infection (UTI) is a bacterial infection of the bladder or lower urinary tract (urethra) that lasts for a long time. Most urinary tract infections occur in the lower urinary tract, which includes the bladder and urethra. The condition occurs when the normally clean lower urinary tract is infected by bacteria and becomes inflamed. Urinary tract infections are very common. Most of the time, symptoms of a urinary tract infection disappear within 24-48 hours after treatment begins. However, if the condition occurs more than twice in 6 months, lasts longer than 2 weeks, or does not respond to usual treatment, it is considered chronic. The elderly are at increased risk for such infections because the bladder doesn't empty fully due to such conditions as benign prostatic hyperplasia, prostatitis, and urethral strictures. Other irritating symptoms may include painful urination (dysuria), which may be a result of a urinary tract infection (UTI) caused by urine being held too long in the bladder. UTI with fever is a sign of potential severe kidney infection (pyelonephritis) and is a more worrisome situation as it may result in permanent damage of the kidney(s). Another type of urinary tract infection is vesicoureteral reflux (VUR). Vesicoureteral reflux is an abnormal backup of urine from the bladder to the kidney(s) that occurs as a means of releasing high pressure within the bladder. A UTI is of particular concern as VUR may place the patient at significant risk for a severe kidney infection by transporting infected bladder urine directly to the kidney(s).

Thus in embodiment, a mammal suffering from chronic urinary tract infection is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the chronic urinary tract infection. In an aspect of this embodiment, a mammal suffering from dysuria is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the dysuria. In an aspect of this embodiment, a mammal suffering from vesicoureteral reflux is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the vesicoureteral reflux.

Other types of urogenital-neurological disorders are disorders associated with prostate disorders. The prostate is a partially glandular and partially fibromuscular organ of the male reproductive system that that produces the fluid that carries sperm during ejaculation. It surrounds the urethra, the tube through which urine passes out of the body. One type of prostate disorder is benign prostatic hyperplasia (BPH). During aging, the prostate tends to enlarge (hypertrophy) and this enlarged prostate is often called benign prostatic hyperplasia (BPH) or benign prostatic hypertrophy. Prostatic enlargement can lead to urethral obstruction and voiding dysfunction because the enlarged gland can press on the urethra. BPH is not cancer, and it does not raise your risk for prostate cancer.

One type of prostate disorder is prostatitis. Prostatitis is an inflammation of the prostate gland. Prostatitis include acute and chronic bacterial prostatitis and inflammation not caused by bacterial infection (abacterial prostatitis). One type of prostate disorder is prostatodynia. Prostatodynia is a type of inflammation of the prostate not due to bacterial infection that may be caused by abnormal nerves or muscles in the region. Prostatodynia is typically a chronic, painful disease. The symptoms (including chills, fever, pain in the lower back and genital area, body aches, burning or painful urination, and the frequent and urgent need to urinate) characteristically go away and then come back without warning.

Thus in embodiment, a mammal suffering from a urogenital-neurological disorder associated with a prostate disorder is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the urogenital-neurological disorder associated with the prostate disorder. In another aspect of this embodiment, a mammal suffering from urogenital-neurological disorder associated with benign prostatic hyperplasia is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the urogenital-neurological disorder associated with benign prostatic hyperplasia. In yet another aspect of this embodiment, a mammal suffering from urogenital-neurological disorder associated with prostatitis is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the urogenital-neurological disorder associated with prostatitis. In still another aspect of this embodiment, a mammal suffering from urogenital-neurological disorder associated with prostatodynia is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the urogenital-neurological disorder associated with prostatodynia.

In another embodiment, a mammal suffering from a prostate disorder is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the prostate disorder. In an aspect of this embodiment, a mammal suffering from benign prostatic hyperplasia is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the benign prostatic hyperplasia. In yet another aspect of this embodiment, a mammal suffering from prostatitis is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the prostatitis. In still another aspect of this embodiment, a mammal suffering from prostatodynia is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the prostatodynia.

Other types of urogenital-neurological disorders are disorders associated with uterine disorders. The uterus is a hollow, muscular pear-shaped female reproductive organ in which the fertilized zygote implants and develops into the fetus. The uterus comprises a corpus made up of two layers of tissue, fundus, isthmus, and cervix located between the urinary bladder and the rectum in the pelvic cavity of female mammals. One type of uterine disorder is endometriosis. Endometriosis is a condition in which the tissue that lines the inside of the uterus (called the endometrium or endometrial lining) is found growing in other areas outside of the uterus (commonly the ovaries, fallopian tubes, outer surface of the uterus, outer surface of the intestines, and nearby structures of the pelvis). This condition often causes severe pain within the lower abdomen and pelvis that may be associated with your periods each month. The symptoms of endometriosis include pain before and during menstrual periods, pain at the time of ovulation, pain during or after sexual activity, heavy or irregular bleeding, fatigue, pain with bowel movements at the time of the period, pain with urination. Another type of uterine disorder is dysmenorrhea. Dysmenorrhea is the pain or discomfort (menstrual cramps) during or just before a menstrual period. There are two types of dysmenorrheal, primary dysmenorrhea and secondary dysmenorrhea. Primary dysmenorrhea is severe, disabling cramps without underlying illness. Symptoms may include backache, leg pain, nausea, vomiting, diarrhea, headache, and dizziness. This kind of dysmenorrhea usually affects young woman within two years of the onset of menstruation and lasts one or two days each month. Secondary dysmenorrhea is cramps caused by another medical problem(s) such as endometriosis (abnormalities in the lining of the uterus), adenomyosis (nonmalignant growth of the endometrium into the muscular layer of the uterus), pelvic inflammatory disease, uterine fibroids, cervical narrowing, uterine malposition, pelvic tumors or an IUD (intra-uterine device). This condition usually occurs in older women.

Thus in embodiment, a mammal suffering from a urogenital-neurological disorder associated with a uterine disorder is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the urogenital-neurological disorder associated with the uterine disorder. In an aspect of this embodiment, a mammal suffering from endometriosis is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the endometriosis. In an aspect of this embodiment, a mammal suffering from dysmenorrhea is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the dysmenorrhea.

Other types of urogenital-neurological disorders are urogenital-neurological disorders associated with neurogenic dysfunction. Thus in embodiment, a mammal suffering from a urogenital-neurological disorder associated with a neurogenic dysfunction is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the urogenital-neurological disorder associated with the neurogenic dysfunction. In an aspect of this embodiment, a mammal suffering from a urogenital-neurological disorder associated with Parkinson's Disease is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the urogenital-neurological disorder associated with Parkinson's Disease. In another aspect of this embodiment, a mammal suffering from a urogenital-neurological disorder associated with multiple sclerosis is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the urogenital-neurological disorder associated with multiple sclerosis. In yet another aspect of this embodiment, a mammal suffering from a urogenital-neurological disorder associated with spina bifida is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symp-

tom associated with the urogenital-neurological disorder associated with spina bifida. In yet another aspect of this embodiment, a mammal suffering from a urogenital-neurological disorder associated with transverse myelitis is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the urogenital-neurological disorder associated with transverse myelitis. In yet another aspect of this embodiment, a mammal suffering from a urogenital-neurological disorder associated with stroke is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the urogenital-neurological disorder associated with stroke. In still another aspect of this embodiment, a mammal suffering from a urogenital-neurological disorder associated with a spinal cord injury is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the urogenital-neurological disorder associated with the spinal cord injury. In still another aspect of this embodiment, a mammal suffering from a urogenital-neurological disorder associated with a spasm reflex is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the urogenital-neurological disorder associated with the spasm reflex. In a further aspect of this embodiment, a mammal suffering from a urogenital-neurological disorder associated with a neurologic lesion of the spinal cord or brain is treated with a composition comprising a therapeutically effective amount of a modified Clostridial toxin where such administration reduces a symptom associated with the urogenital-neurological disorder associated with the neurologic lesion of the spinal cord or brain.

Aspects of the present invention provide, in part, a mammal. A mammal includes a human, and a human can be a patient. Other aspects of the present invention provide, in part, an individual. An individual includes a human, and a human can be a patient.

Aspects of the present invention provide, in part, administering a composition comprising a modified Clostridial toxin. As used herein, the term "administering" means any delivery mechanism that provides a composition comprising a modified Clostridial toxin to a patient that potentially results in a clinically, therapeutically, or experimentally beneficial result. A modified Clostridial toxin can be delivered to a patient using a cellular uptake approach where a modified Clostridial toxin is delivered intracellular or a gene therapy approach where a modified Clostridial toxin is express derived from precursor RNAs expressed from an expression vectors.

A composition comprising a modified Clostridial toxin as disclosed in the present specification can be administered to a mammal using a cellular uptake approach. Administration of a composition comprising a modified Clostridial toxin using a cellular uptake approach comprise a variety of enteral or parenteral approaches including, without limitation, oral administration in any acceptable form, such as, e.g., tablet, liquid, capsule, powder, or the like; topical administration in any acceptable form, such as, e.g., drops, spray, creams, gels or ointments; intravascular administration in any acceptable form, such as, e.g., intravenous bolus injection, intravenous infusion, intra-arterial bolus injection, intra-arterial infusion and catheter instillation into the vasculature; per- and intratissue administration in any acceptable form, such as, e.g., intraperitoneal injection, intramuscular injection, subcutaneous injection, subcutaneous infusion, intraocular injection, retinal injection, or sub-retinal injection or epidural injection; intravesicular administration in any acceptable form, such as, e.g., catheter instillation; and by placement device, such as, e.g., an implant, a patch, a pellet, a catheter, an osmotic pump, a suppository, a bioerodible delivery system, a non-bioerodible delivery system or another implanted extended or slow release system. An exemplary list of biodegradable polymers and methods of use are described in, e.g., *Handbook of Biodegradable Polymers* (Abraham J. Domb et al., eds., Overseas Publishers Association, 1997).

A composition comprising a modified Clostridial toxin can be administered to a mammal by a variety of methods known to those of skill in the art, including, but not restricted to, encapsulation in liposomes, by ionophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres, or by proteinaceous vectors. Delivery mechanisms for administering a composition comprising a modified Clostridial toxin to a patient are described in, e.g., Leonid Beigelman et al., Compositions for the Delivery of Negatively Charged Molecules, U.S. Pat. No. 6,395,713 (May 28, 2002); and Achim Aigner, *Delivery Systems for the Direct Application of siRNAs to Induce RNA Interference (RNAi) in vivo,* 2006 (716559) J. Biomed. Biotech. 1-15 (2006); *Controlled Drug Delivery: Designing Technologies for the Future* (Kinam Park & Randy J. Mrsny eds., American Chemical Association, 2000); Vernon G. Wong & Mae W. L. Hu, Methods for Treating Inflammation-mediated Conditions of the Eye, U.S. Pat. No. 6,726,918 (Apr. 27, 2004); David A. Weber et al., Methods and Apparatus for Delivery of Ocular Implants, U.S. Patent Publication No. US2004/0054374 (Mar. 18, 2004); Thierry Nivaggioli et al., Biodegradable Ocular Implant, U.S. Patent Publication No. US2004/0137059 (Jul. 15, 2004); Patrick M. Hughes et al., Anti-Angiogenic Sustained Release Intraocular Implants and Related Methods, U.S. patent application Ser. No. 11/364,687 (Feb. 27, 2006); and Patrick M. Hughes et al., Sustained Release Intraocular Drug Delivery Systems, U.S. Patent Publication 2006/0182783 (Aug. 17, 2006), each of which is hereby incorporated by reference in its entirety.

A composition comprising a modified Clostridial toxin as disclosed in the present specification can also be administered to a patient using a gene therapy approach by expressing a modified Clostridial toxin within in a cell manifesting a nerve-based etiology that contributes to a urogenital-neurological disorder. A modified Clostridial toxin can be expressed from nucleic acid molecules operably-linked to an expression vector, see, e.g., P. D. Good et al., *Expression of Small, Therapeutic RNAs in Human Cell Nuclei,* 4(1) Gene Ther. 45-54 (1997); James D. Thompson, Polymerase III-based expression of therapeutic RNAs, U.S. Pat. No. 6,852,535 (Feb. 8, 2005); Maciej Wiznerowicz et al., *Tuning Silence: Conditional Systems for RNA Interference,* 3(9) Nat. Methods 682-688m (2006); Ola Snøve and John J. Rossi, *Expressing Short Hairpin RNAi in vivo,* 3(9) Nat. Methods 689-698 (2006); and Charles X. Li et al., *Delivery of RNA Interference,* 5(18) Cell Cycle 2103-2109 (2006). A person of ordinary skill in the art would realize that any modified Clostridial toxin can be expressed in eukaryotic cells using an appropriate expression vector.

Expression vectors capable of expressing a modified Clostridial toxin can provide persistent or stable expression of the modified Clostridial toxin in a cell manifesting a nerve-based etiology that contributes to a urogenital-neurological disorder. Alternatively, expression vectors capable of expressing a modified Clostridial toxin can provide for transient expression of the modified Clostridial toxin in a cell

manifesting a nerve-based etiology that contributes to a urogenital-neurological disorder. Such transiently expressing vectors can be repeatedly administered as necessary. A modified Clostridial toxin-expressing vectors can be administered by a delivery mechanism and route of administration discussed above, by administration to target cells ex-planted from a patient followed by reintroduction into the patient, or by any other means that would allow for introduction into the desired target cell, see, e.g., Larry A. Couture and Dan T. Stinchcomb, *Anti-gene Therapy: The Use of Ribozymes to Inhibit Gene Function,* 12(12) Trends Genet. 510-515 (1996).

The actual delivery mechanism used to administer a composition comprising a modified Clostridial toxin to a mammal can be determined by a person of ordinary skill in the art by taking into account factors, including, without limitation, the type of urogenital-neurological disorder, the location of the urogenital-neurological disorder, the cause of the urogenital-neurological disorder, the severity of the urogenital-neurological disorder, the degree of relief desired, the duration of relief desired, the particular modified Clostridial toxin used, the rate of excretion of the modified Clostridial toxin used, the pharmacodynamics of the modified Clostridial toxin used, the nature of the other compounds to be included in the composition, the particular route of administration, the particular characteristics, history and risk factors of the patient, such as, e.g., age, weight, general health and the like, or any combination thereof.

In an embodiment, a composition comprising a modified Clostridial toxin is administered to the site to be treated by injection. In aspects of this embodiment, injection of a composition comprising a modified Clostridial toxin is by, e.g., intramuscular injection, subdermal injection, or dermal injection. In aspects of this embodiment, injection of a composition comprising a modified Clostridial toxin is into the lower urinary tract, including the bladder wall, the urinary sphincter or bladder neck.

A composition comprising a modified Clostridial toxin can be administered to a mammal using a variety of routes. Routes of administration suitable for a method of treating an urogenital-neurological disorder as disclosed in the present specification include both local and systemic administration. Local administration results in significantly more delivery of a composition to a specific location as compared to the entire body of the mammal, whereas, systemic administration results in delivery of a composition to essentially the entire body of the patient. Routes of administration suitable for a method of treating an urogenital-neurological disorder as disclosed in the present specification also include both central and peripheral administration. Central administration results in delivery of a composition to essentially the central nervous system of the patient and includes, e.g., intrathecal administration, epidural administration as well as a cranial injection or implant. Peripheral administration results in delivery of a composition to essentially any area of a patient outside of the central nervous system and encompasses any route of administration other than direct administration to the spine or brain. The actual route of administration of a composition comprising a modified Clostridial toxin used in a mammal can be determined by a person of ordinary skill in the art by taking into account factors, including, without limitation, the type of urogenital-neurological disorder, the location of the urogenital-neurological disorder, the cause of the urogenital-neurological disorder, the severity of the urogenital-neurological disorder, the degree of relief desired, the duration of relief desired, the particular modified Clostridial toxin used, the rate of excretion of the modified Clostridial toxin used, the pharmacodynamics of the modified Clostridial toxin used, the nature of the other compounds to be included in the composition, the particular route of administration, the particular characteristics, history and risk factors of the mammal, such as, e.g., age, weight, general health and the like, or any combination thereof.

In an embodiment, a composition comprising a modified Clostridial toxin is administered systemically to a mammal. In another embodiment, a composition comprising a modified Clostridial toxin is administered locally to a mammal. In an aspect of this embodiment, a composition comprising a modified Clostridial toxin is administered to the bladder of a mammal. In another aspect of this embodiment, a composition comprising a modified Clostridial toxin is administered to the prostate of a mammal. In another aspect of this embodiment, a composition comprising a modified Clostridial toxin is administered to the uterus of a mammal.

Aspects of the present invention provide, in part, administering a therapeutically effective amount of a composition comprising a modified Clostridial toxin. As used herein, the term "therapeutically effective amount" is synonymous with "therapeutically effective dose" and when used in reference to treating an urogenital-neurological disorder means the minimum dose of a modified Clostridial toxin necessary to achieve the desired therapeutic effect and includes a dose sufficient to reduce a symptom associated with an urogenital-neurological disorder. In aspects of this embodiment, a therapeutically effective amount of a composition comprising a modified Clostridial toxin reduces a symptom associated with an urogenital-neurological disorder by, e.g., at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 100%. In other aspects of this embodiment, a therapeutically effective amount of a composition comprising a modified Clostridial toxin reduces a symptom associated with an urogenital-neurological disorder by, e.g., at most 30%, at most 40%, at most 50%, at most 60%, at most 70%, at most 80%, at most 90% or at most 100%. In yet other aspects of this embodiment, a therapeutically effective amount of a composition comprising a modified Clostridial toxin reduces a symptom associated with an urogenital-neurological disorder by, e.g., about 10% to about 100%, about 10% to about 90%, about 10% to about 80%, about 10% to about 70%, about 10% to about 60%, about 10% to about 50%, about 10% to about 40%, about 20% to about 100%, about 20% to about 90%, about 20% to about 80%, about 20% to about 20%, about 20% to about 60%, about 20% to about 50%, about 20% to about 40%, about 30% to about 100%, about 30% to about 90%, about 30% to about 80%, about 30% to about 70%, about 30% to about 60%, or about 30% to about 50%. As used herein, the term "about" when qualifying a value of a stated item, number, percentage, or term refers to a range of plus or minus ten percent of the value of the stated item, percentage, parameter, or term. In still other aspects of this embodiment, a therapeutically effective amount of the modified Clostridial toxin is the dosage sufficient to inhibit neuronal activity for, e.g., at least one week, at least one month, at least two months, at least three months, at least four months, at least five months, at least six months, at least seven months, at least eight months, at least nine months, at least ten months, at least eleven months, or at least twelve months.

The actual therapeutically effective amount of a composition comprising a modified Clostridial toxin to be administered to a mammal can be determined by a person of ordinary skill in the art by taking into account factors, including, without limitation, the type of urogenital-neurological disorder, the location of the urogenital-neurological disorder, the cause of the urogenital-neurological disorder, the severity of the

urogenital-neurological disorder, the degree of relief desired, the duration of relief desired, the particular modified Clostridial toxin used, the rate of excretion of the modified Clostridial toxin used, the pharmacodynamics of the modified Clostridial toxin used, the nature of the other compounds to be included in the composition, the particular route of administration, the particular characteristics, history and risk factors of the patient, such as, e.g., age, weight, general health and the like, or any combination thereof. Additionally, where repeated administration of a composition comprising a modified Clostridial toxin is used, the actual effect amount of a composition comprising a modified Clostridial toxin will further depend upon factors, including, without limitation, the frequency of administration, the half-life of the composition comprising a modified Clostridial toxin, or any combination thereof. In is known by a person of ordinary skill in the art that an effective amount of a composition comprising a modified Clostridial toxin can be extrapolated from in vitro assays and in vivo administration studies using animal models prior to administration to humans. Wide variations in the necessary effective amount are to be expected in view of the differing efficiencies of the various routes of administration. For instance, oral administration generally would be expected to require higher dosage levels than administration by intravenous or intravitreal injection. Variations in these dosage levels can be adjusted using standard empirical routines of optimization, which are well-known to a person of ordinary skill in the art. The precise therapeutically effective dosage levels and patterns are preferably determined by the attending physician in consideration of the above-identified factors.

As a non-limiting example, when administering a composition comprising a modified Clostridial toxin to a mammal, a therapeutically effective amount generally is in the range of about 1 fg to about 3.0 mg. In aspects of this embodiment, an effective amount of a composition comprising a modified Clostridial toxin can be, e.g., about 100 fg to about 3.0 mg, about 100 pg to about 3.0 mg, about 100 ng to about 3.0 mg, or about 100 μg to about 3.0 mg. In other aspects of this embodiment, an effective amount of a composition comprising a modified Clostridial toxin can be, e.g., about 100 fg to about 750 μg, about 100 pg to about 750 μg, about 100 ng to about 750 μg, or about 1 μg to about 750 μg. In yet other aspects of this embodiment, a therapeutically effective amount of a composition comprising a modified Clostridial toxin can be, e.g., at least 1 fg, at least 250 fg, at least 500 fg, at least 750 fg, at least 1 pg, at least 250 pg, at least 500 pg, at least 750 pg, at least 1 ng, at least 250 ng, at least 500 ng, at least 750 ng, at least 1 μg, at least 250 μg, at least 500 μg, at least 750 μg, or at least 1 mg. In still other aspects of this embodiment, a therapeutically effective amount of a composition comprising a modified Clostridial toxin can be, e.g., at most 1 fg, at most 250 fg, at most 500 fg, at most 750 fg, at most 1 pg, at most 250 pg, at most 500 pg, at most 750 pg, at most 1 ng, at most 250 ng, at most 500 ng, at most 750 ng, at most 1 μg, at least 250 μg, at most 500 μg, at most 750 μg, or at most 1 mg.

As another non-limiting example, when administering a composition comprising a modified Clostridial toxin to a mammal, a therapeutically effective amount generally is in the range of about 0.00001 mg/kg to about 3.0 mg/kg. In aspects of this embodiment, an effective amount of a composition comprising a modified Clostridial toxin can be, e.g., about 0.0001 mg/kg to about 0.001 mg/kg, about 0.03 mg/kg to about 3.0 mg/kg, about 0.1 mg/kg to about 3.0 mg/kg, or about 0.3 mg/kg to about 3.0 mg/kg. In yet other aspects of this embodiment, a therapeutically effective amount of a composition comprising a modified Clostridial toxin can be, e.g., at least 0.00001 mg/kg, at least 0.0001 mg/kg, at least 0.001 mg/kg, at least 0.01 mg/kg, at least 0.1 mg/kg, or at least 1 mg/kg. In yet other aspects of this embodiment, a therapeutically effective amount of a composition comprising a modified Clostridial toxin can be, e.g., at most 0.00001 mg/kg, at most 0.0001 mg/kg, at most 0.001 mg/kg, at most 0.01 mg/kg, at most 0.1 mg/kg, or at most 1 mg/kg.

Dosing can be single dosage or cumulative (serial dosing), and can be readily determined by one skilled in the art. For instance, treatment of an urogenital-neurological disorder may comprise a one-time administration of an effective dose of a composition comprising a modified Clostridial toxin. As a non-limiting example, an effective dose of a composition comprising a modified Clostridial toxin can be administered once to a patient, e.g., as a single injection or deposition at or near the site exhibiting a symptom of an urogenital-neurological disorder. Alternatively, treatment of an urogenital-neurological disorder may comprise multiple administrations of an effective dose of a composition comprising a modified Clostridial toxin carried out over a range of time periods, such as, e.g., daily, once every few days, weekly, monthly or yearly. As a non-limiting example, a composition comprising a modified Clostridial toxin can be administered once or twice yearly to a mammal. The timing of administration can vary from mammal to mammal, depending upon such factors as the severity of a mammal's symptoms. For example, an effective dose of a composition comprising a modified Clostridial toxin can be administered to a mammal once a month for an indefinite period of time, or until the patient no longer requires therapy. A person of ordinary skill in the art will recognize that the condition of the mammal can be monitored throughout the course of treatment and that the effective amount of a composition comprising a modified Clostridial toxin that is administered can be adjusted accordingly.

A composition comprising a modified Clostridial toxin as disclosed in the present specification can also be administered to a mammal in combination with other therapeutic compounds to increase the overall therapeutic effect of the treatment. The use of multiple compounds to treat an indication can increase the beneficial effects while reducing the presence of side effects.

Aspects of the present invention can also be described as follows:

1. A method of treating urogenital-neurological disorder in a mammal, the method comprising the step of administering to the mammal in need thereof a therapeutically effective amount of a composition including a modified Clostridial toxin comprising an opioid peptide binding domain, a Clostridial toxin translocation domain and a Clostridial toxin enzymatic domain, wherein administration of the composition reduces a symptom of the urogenital-neurological disorder, thereby treating the mammal.
2. The method of 1, wherein the modified Clostridial toxin comprises a linear amino-to-carboxyl single polypeptide order of 1) the Clostridial toxin enzymatic domain, the Clostridial toxin translocation domain, the opioid peptide binding domain, 2) the Clostridial toxin enzymatic domain, the opioid peptide binding domain, the Clostridial toxin translocation domain, 3) the opioid peptide binding domain, the Clostridial toxin translocation domain, and the Clostridial toxin enzymatic domain, 4) the opioid peptide binding domain, the Clostridial toxin enzymatic domain, the Clostridial toxin translocation domain, 5) the Clostridial toxin translocation domain, the Clostridial toxin enzymatic domain and the opioid peptide binding

domain, or 6) the Clostridial toxin translocation domain, the opioid peptide binding domain and the Clostridial toxin enzymatic domain.

3. The method of 1, wherein the opioid peptide binding domain is an enkephalin, a BAM22 peptide, an endomorphin, an endorphin, a dynorphin, a nociceptin or a hemorphin.

4. The method of 3, wherein the enkephalin is a Leu-enkephalin, a Met-enkephalin, a Met-enkephalin MRGL or a Met-enkephalin MRF.

5. The method of 3, wherein the enkephalin comprises SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54 or SEQ ID NO: 55.

6. The method of 3, wherein the BAM22 peptide is a BAM22 peptide (1-12), a BAM22 peptide (6-22), a BAM22 peptide (8-22) or a BAM22 peptide (1-22)

7. The method of 3, wherein the BAM22 peptide comprises amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 56; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 57; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 58; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 59; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 60 or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 61.

8. The method of 3, wherein the endomorphin is an endomorphin-1 or an endomorphin-2.

9. The method of 3, wherein the endomorphin comprises SEQ ID NO: 62 or SEQ ID NO: 63.

10. The method of 3, wherein the endorphin an endorphin-α, a neoendorphin-α, an endorphin-β, a neoendorphin-β or an endorphin-γ.

11. The method of 3, wherein the endorphin comprises SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68 or SEQ ID NO: 69.

12. The method of 3, wherein the dynorphin is a dynorphin A, a dynorphin B (leumorphin) or a rimorphin.

13. The method of 3, wherein the dynorphin comprises SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99 or SEQ ID NO: 100.

14. The method of 3, wherein the nociceptin is a nociceptin RK, a nociceptin, a neuropeptide 1, a neuropeptide 2 or a neuropeptide 3.

15. The method of 3, wherein the nociceptin comprises SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109 or SEQ ID NO: 110.

16. The method of 1, wherein the Clostridial toxin translocation domain is a BoNT/A translocation domain, a BoNT/B translocation domain, a BoNT/C1 translocation domain, a BoNT/D translocation domain, a BoNT/E translocation domain, a BoNT/F translocation domain, a BoNT/G translocation domain, a TeNT translocation domain, a BaNT translocation domain, or a BuNT translocation domain.

17. The method of 1, wherein the Clostridial toxin enzymatic domain is a BoNT/A enzymatic domain, a BoNT/B enzymatic domain, a BoNT/C1 enzymatic domain, a BoNT/D enzymatic domain, a BoNT/E enzymatic domain, a BoNT/F enzymatic domain, a BoNT/G enzymatic domain, a TeNT enzymatic domain, a BaNT enzymatic domain, or a BuNT enzymatic domain.

18. The method of 1, wherein the urogenital-neurological disorder is urinary incontinence, overactive bladder, detrusor dysfunction, lower urinary tract dysfunction, urinary retention, urinary hesitancy, polyuria, nocturia, chronic urinary tract infection, an urogenital disorder associated with a prostate disorder, an urogenital disorder associated with a uterine disorder, or an urogenital disorder associated with a neurogenic dysfunction.

19. The method of 18, wherein the urinary incontinence is an urge urinary incontinence, a stress urinary incontinence, an overflow urinary incontinence, a mixed urinary incontinence, or a continuous urinary incontinence.

20. The method of 18, wherein the detrusor dysfunction is a detrusor overactivity, a detrusor instability, or a detrusor-sphincter dyssynergia.

21. The method of 18, wherein the urogenital disorder associated with a prostate disorder is an urogenital disorder associated with benign prostatic hyperplasia, an urogenital disorder associated with prostatitis, or an urogenital disorder associated with prostatodynia.

22. The method of 18, wherein the urogenital disorder associated with a neurogenic dysfunction is an urogenital disorder associated with Parkinson's Disease, an urogenital disorder associated with multiple sclerosis, an urogenital disorder associated with spina bifida, an urogenital disorder associated with transverse myelitis, an urogenital disorder associated with stroke, an urogenital disorder associated with a spinal cord injury, an urogenital disorder associated with a spasm reflex, an urogenital disorder associated with a neurologic lesion of the spinal cord, or an urogenital disorder associated with a neurologic lesion of the brain.

23. A method of treating urogenital-neurological disorder in a mammal, the method comprising the step of administering to the mammal in need thereof a therapeutically effective amount of a composition including a modified Clostridial toxin comprising an opioid peptide binding domain, a Clostridial toxin translocation domain, a Clostridial toxin enzymatic domain, and an exogenous protease cleavage site, wherein administration of the composition reduces a symptom of the urogenital-neurological disorder, thereby treating the mammal.

24. The method of 23, wherein the modified Clostridial toxin comprises a linear amino-to-carboxyl single polypeptide order of 1) the Clostridial toxin enzymatic domain, the exogenous protease cleavage site, the Clostridial toxin translocation domain, the opioid peptide binding domain, 2) the Clostridial toxin enzymatic domain, the exogenous protease cleavage site, the opioid peptide binding domain, the Clostridial toxin translocation domain, 3) the opioid peptide binding domain, the Clostridial toxin translocation domain, the exogenous protease cleavage site and the Clostridial toxin enzymatic domain, 4) the opioid peptide binding domain, the Clostridial toxin enzymatic domain, the exogenous protease cleavage site, the Clostridial toxin translocation domain, 5) the Clostridial toxin translocation domain, the exogenous protease cleavage site, the Clostridial toxin enzymatic domain and the opioid peptide binding domain, or 6) the Clostridial toxin translocation domain, the exogenous protease cleavage site, the opioid peptide binding domain and the Clostridial toxin enzymatic domain.

25. The method of 23, wherein the opioid peptide binding domain is an enkephalin, a BAM22 peptide, an endomorphin, an endorphin, a dynorphin, a nociceptin or a hemorphin.
26. The method of 25, wherein the enkephalin is a Leu-enkephalin, a Met-enkephalin, a Met-enkephalin MRGL or a Met-enkephalin MRF.
27. The method of 25, wherein the enkephalin comprises SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54 or SEQ ID NO: 55.
28. The method of 25, wherein the BAM22 peptide is a BAM22 peptide (1-12), a BAM22 peptide (6-22), a BAM22 peptide (8-22) or a BAM22 peptide (1-22)
29. The method of 25, wherein the BAM22 peptide comprises amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 56; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 57; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 58; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 59; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 60 or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 61.
30. The method of 25, wherein the endomorphin is an endomorphin-1 or an endomorphin-2.
31. The method of 25, wherein the endomorphin comprises SEQ ID NO: 62 or SEQ ID NO: 63.
32. The method of 25, wherein the endorphin an endorphin-α, a neoendorphin-α, an endorphin-β, a neoendorphin-β or an endorphin-γ.
33. The method of 25, wherein the endorphin comprises SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68 or SEQ ID NO: 69.
34. The method of 25, wherein the dynorphin is a dynorphin A, a dynorphin B (leumorphin) or a rimorphin.
35. The method of 25, wherein the dynorphin comprises SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99 or SEQ ID NO: 100.
36. The method of 25, wherein the nociceptin is a nociceptin RK, a nociceptin, a neuropeptide 1, a neuropeptide 2 or a neuropeptide 3.
37. The method of 25, wherein the nociceptin comprises SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109 or SEQ ID NO: 110.
38. The method of 23, wherein the Clostridial toxin translocation domain is a BoNT/A translocation domain, a BoNT/B translocation domain, a BoNT/C1 translocation domain, a BoNT/D translocation domain, a BoNT/E translocation domain, a BoNT/F translocation domain, a BoNT/G translocation domain, a TeNT translocation domain, a BaNT translocation domain, or a BuNT translocation domain.
39. The method of 23, wherein the Clostridial toxin enzymatic domain is a BoNT/A enzymatic domain, a BoNT/B enzymatic domain, a BoNT/C1 enzymatic domain, a BoNT/D enzymatic domain, a BoNT/E enzymatic domain, a BoNT/F enzymatic domain, a BoNT/G enzymatic domain, a TeNT enzymatic domain, a BaNT enzymatic domain, or a BuNT enzymatic domain.
40. The method of 23, wherein the exogenous protease cleavage site is a plant papain cleavage site, an insect papain cleavage site, a crustacian papain cleavage site, an enterokinase cleavage site, a human rhinovirus 3C protease cleavage site, a human enterovirus 3C protease cleavage site, a tobacco etch virus protease cleavage site, a Tobacco Vein Mottling Virus cleavage site, a subtilisin cleavage site, a hydroxylamine cleavage site, or a Caspase 3 cleavage site.
41. The method of 23, wherein the urogenital-neurological disorder is urinary incontinence, overactive bladder, detrusor dysfunction, lower urinary tract dysfunction, urinary retention, urinary hesitancy, polyuria, nocturia, chronic urinary tract infection, an urogenital disorder associated with a prostate disorder, an urogenital disorder associated with a uterine disorder, or an urogenital disorder associated with a neurogenic dysfunction.
42. The method of 41, wherein the urinary incontinence is an urge urinary incontinence, a stress urinary incontinence, an overflow urinary incontinence, a mixed urinary incontinence, or a continuous urinary incontinence.
43. The method of 41, wherein the detrusor dysfunction is a detrusor overactivity, a detrusor instability, or a detrusor-sphincter dyssynergia.
44. The method of 41, wherein the urogenital disorder associated with a prostate disorder is an urogenital disorder associated with benign prostatic hyperplasia, an urogenital disorder associated with prostatitis, or an urogenital disorder associated with prostatodynia.
45. The method of 41, wherein the urogenital disorder associated with a neurogenic dysfunction is an urogenital disorder associated with Parkinson's Disease, an urogenital disorder associated with multiple sclerosis, an urogenital disorder associated with spina bifida, an urogenital disorder associated with transverse myelitis, an urogenital disorder associated with stroke, an urogenital disorder associated with a spinal cord injury, an urogenital disorder associated with a spasm reflex, an urogenital disorder associated with a neurologic lesion of the spinal cord, or an urogenital disorder associated with a neurologic lesion of the brain.
46. A use of a modified Clostridial toxin in the manufacturing a medicament for treating urogenital-neurological disorder in a mammal in need thereof, wherein the modified Clostridial toxin comprising an opioid peptide binding domain, a Clostridial toxin translocation domain and a Clostridial toxin enzymatic domain and wherein administration of a therapeutically effective amount of the medicament to the mammal reduces a symptom of the urogenital-neurological disorder, thereby treating the mammal.
47. The use of 46, wherein the modified Clostridial toxin comprises a linear amino-to-carboxyl single polypeptide order of 1) the Clostridial toxin enzymatic domain, the Clostridial toxin translocation domain, the opioid peptide binding domain, 2) the Clostridial toxin enzymatic domain, the opioid peptide binding domain, the Clostridial toxin translocation domain, 3) the opioid peptide binding domain, the Clostridial toxin translocation domain, and the Clostridial toxin enzymatic domain, 4) the opioid peptide binding domain, the Clostridial toxin enzymatic domain, the Clostridial toxin translocation domain, 5) the Clostridial toxin translocation domain, the Clostridial toxin enzymatic domain and the opioid peptide binding

domain, or 6) the Clostridial toxin translocation domain, the opioid peptide binding domain and the Clostridial toxin enzymatic domain.

48. The use of 46, wherein the opioid peptide binding domain is an enkephalin, a BAM22 peptide, an endomorphin, an endorphin, a dynorphin, a nociceptin or a hemorphin.

49. The method of 48, wherein the enkephalin is a Leu-enkephalin, a Met-enkephalin, a Met-enkephalin MRGL or a Met-enkephalin MRF.

50. The method of 48, wherein the enkephalin comprises SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54 or SEQ ID NO: 55.

51. The method of 48, wherein the BAM22 peptide is a BAM22 peptide (1-12), a BAM22 peptide (6-22), a BAM22 peptide (8-22) or a BAM22 peptide (1-22)

52. The method of 48, wherein the BAM22 peptide comprises amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 56; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 57; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 58; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 59; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 60 or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 61.

53. The method of 48, wherein the endomorphin is an endomorphin-1 or an endomorphin-2.

54. The method of 48, wherein the endomorphin comprises SEQ ID NO: 62 or SEQ ID NO: 63.

55. The method of 48, wherein the endorphin an endorphin-α, a neoendorphin-α, an endorphin-β, a neoendorphin-β or an endorphin-γ.

56. The method of 48, wherein the endorphin comprises SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68 or SEQ ID NO: 69.

57. The method of 48, wherein the dynorphin is a dynorphin A, a dynorphin B (leumorphin) or a rimorphin.

58. The method of 48, wherein the dynorphin comprises SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99 or SEQ ID NO: 100.

59. The method of 48, wherein the nociceptin is a nociceptin RK, a nociceptin, a neuropeptide 1, a neuropeptide 2 or a neuropeptide 3.

60. The method of 48, wherein the nociceptin comprises SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109 or SEQ ID NO: 110.

61. The use of 46, wherein the Clostridial toxin translocation domain is a BoNT/A translocation domain, a BoNT/B translocation domain, a BoNT/C1 translocation domain, a BoNT/D translocation domain, a BoNT/E translocation domain, a BoNT/F translocation domain, a BoNT/G translocation domain, a TeNT translocation domain, a BaNT translocation domain, or a BuNT translocation domain.

62. The use of 46, wherein the Clostridial toxin enzymatic domain is a BoNT/A enzymatic domain, a BoNT/B enzymatic domain, a BoNT/C1 enzymatic domain, a BoNT/D enzymatic domain, a BoNT/E enzymatic domain, a BoNT/F enzymatic domain, a BoNT/G enzymatic domain, a TeNT enzymatic domain, a BaNT enzymatic domain, or a BuNT enzymatic domain.

63. The use of 46, wherein the urogenital-neurological disorder is urinary incontinence, overactive bladder, detrusor dysfunction, lower urinary tract dysfunction, urinary retention, urinary hesitancy, polyuria, nocturia, chronic urinary tract infection, an urogenital disorder associated with a prostate disorder, an urogenital disorder associated with a uterine disorder, or an urogenital disorder associated with a neurogenic dysfunction.

64. The use of 63, wherein the urinary incontinence is an urge urinary incontinence, a stress urinary incontinence, an overflow urinary incontinence, a mixed urinary incontinence, or a continuous urinary incontinence.

65. The use of 63, wherein the detrusor dysfunction is a detrusor overactivity, a detrusor instability, or a detrusor-sphincter dyssynergia.

66. The use of 63, wherein the urogenital disorder associated with a prostate disorder is an urogenital disorder associated with benign prostatic hyperplasia, an urogenital disorder associated with prostatitis, or an urogenital disorder associated with prostatodynia.

67. The use of 63, wherein the urogenital disorder associated with a neurogenic dysfunction is an urogenital disorder associated with Parkinson's Disease, an urogenital disorder associated with multiple sclerosis, an urogenital disorder associated with spina bifida, an urogenital disorder associated with transverse myelitis, an urogenital disorder associated with stroke, an urogenital disorder associated with a spinal cord injury, an urogenital disorder associated with a spasm reflex, an urogenital disorder associated with a neurologic lesion of the spinal cord, or an urogenital disorder associated with a neurologic lesion of the brain.

68. A use of a modified Clostridial toxin in the manufacturing a medicament for treating urogenital-neurological disorder in a mammal in need thereof, wherein the modified Clostridial toxin comprising an opioid peptide binding domain, a Clostridial toxin translocation domain and a Clostridial toxin enzymatic domain, and an exogenous protease cleavage site and wherein administration of a therapeutically effective amount of the medicament to the mammal reduces a symptom of the urogenital-neurological disorder, thereby treating the mammal.

69. The use of 68, wherein the modified Clostridial toxin comprises a linear amino-to-carboxyl single polypeptide order of 1) the Clostridial toxin enzymatic domain, the exogenous protease cleavage site, the Clostridial toxin translocation domain, the opioid peptide binding domain, 2) the Clostridial toxin enzymatic domain, the exogenous protease cleavage site, the opioid peptide binding domain, the Clostridial toxin translocation domain, 3) the opioid peptide binding domain, the Clostridial toxin translocation domain, the exogenous protease cleavage site and the Clostridial toxin enzymatic domain, 4) the opioid peptide binding domain, the Clostridial toxin enzymatic domain, the exogenous protease cleavage site, the Clostridial toxin translocation domain, 5) the Clostridial toxin translocation domain, the exogenous protease cleavage site, the Clostridial toxin enzymatic domain and the opioid peptide binding domain, or 6) the Clostridial toxin translocation domain, the exogenous protease cleavage site, the opioid peptide binding domain and the Clostridial toxin enzymatic domain.

70. The use of 69, wherein the opioid peptide binding domain is an enkephalin, a BAM22 peptide, an endomorphin, an endorphin, a dynorphin, a nociceptin or a hemorphin.

71. The method of 70, wherein the enkephalin is a Leu-enkephalin, a Met-enkephalin, a Met-enkephalin MRGL or a Met-enkephalin MRF.
72. The method of 70, wherein the enkephalin comprises SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54 or SEQ ID NO: 55.
73. The method of 70, wherein the BAM22 peptide is a BAM22 peptide (1-12), a BAM22 peptide (6-22), a BAM22 peptide (8-22) or a BAM22 peptide (1-22)
74. The method of 70, wherein the BAM22 peptide comprises amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 56; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 57; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 58; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 59; amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 60 or amino acids 1-12, amino acids 6-22, amino acids 8-22 or amino acids 1-22 of SEQ ID NO: 61.
75. The method of 70, wherein the endomorphin is an endomorphin-1 or an endomorphin-2.
76. The method of 70, wherein the endomorphin comprises SEQ ID NO: 62 or SEQ ID NO: 63.
77. The method of 70, wherein the endorphin an endorphin-α, a neoendorphin-α, an endorphin-β, a neoendorphin-β or an endorphin-γ.
78. The method of 70, wherein the endorphin comprises SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68 or SEQ ID NO: 69.
79. The method of 70, wherein the dynorphin is a dynorphin A, a dynorphin B (leumorphin) or a rimorphin.
80. The method of 70, wherein the dynorphin comprises SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99 or SEQ ID NO: 100.
81. The method of 70, wherein the nociceptin is a nociceptin RK, a nociceptin, a neuropeptide 1, a neuropeptide 2 or a neuropeptide 3.
82. The method of 70, wherein the nociceptin comprises SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109 or SEQ ID NO: 110.
83. The use of 68, wherein the Clostridial toxin translocation domain is a BoNT/A translocation domain, a BoNT/B translocation domain, a BoNT/C1 translocation domain, a BoNT/D translocation domain, a BoNT/E translocation domain, a BoNT/F translocation domain, a BoNT/G translocation domain, a TeNT translocation domain, a BaNT translocation domain, or a BuNT translocation domain.
84. The use of 68, wherein the Clostridial toxin enzymatic domain is a BoNT/A enzymatic domain, a BoNT/B enzymatic domain, a BoNT/C1 enzymatic domain, a BoNT/D enzymatic domain, a BoNT/E enzymatic domain, a BoNT/F enzymatic domain, a BoNT/G enzymatic domain, a TeNT enzymatic domain, a BaNT enzymatic domain, or a BuNT enzymatic domain.
85. The use of 68, wherein the urogenital-neurological disorder is urinary incontinence, overactive bladder, detrusor dysfunction, lower urinary tract dysfunction, urinary retention, urinary hesitancy, polyuria, nocturia, chronic urinary tract infection, an urogenital disorder associated with a prostate disorder, an urogenital disorder associated with a uterine disorder, or an urogenital disorder associated with a neurogenic dysfunction.
86. The use of 85, wherein the urinary incontinence is an urge urinary incontinence, a stress urinary incontinence, an overflow urinary incontinence, a mixed urinary incontinence, or a continuous urinary incontinence.
87. The use of 85, wherein the detrusor dysfunction is a detrusor overactivity, a detrusor instability, or a detrusor-sphincter dyssynergia.
88. The use of 85, wherein the urogenital disorder associated with a prostate disorder is an urogenital disorder associated with benign prostatic hyperplasia, an urogenital disorder associated with prostatitis, or an urogenital disorder associated with prostatodynia.
89. The use of 85, wherein the urogenital disorder associated with a neurogenic dysfunction is an urogenital disorder associated with Parkinson's Disease, an urogenital disorder associated with multiple sclerosis, an urogenital disorder associated with spina bifida, an urogenital disorder associated with transverse myelitis, an urogenital disorder associated with stroke, an urogenital disorder associated with a spinal cord injury, an urogenital disorder associated with a spasm reflex, an urogenital disorder associated with a neurologic lesion of the spinal cord, or an urogenital disorder associated with a neurologic lesion of the brain.
90. A use of a modified Clostridial toxin for treating urogenital-neurological disorder in a mammal in need thereof, the use comprising the step of administering to the mammal in need thereof a therapeutically effective amount of a composition, wherein the modified Clostridial toxin comprising an opioid peptide binding domain, a Clostridial toxin translocation domain and a Clostridial toxin enzymatic domain and wherein administration of the composition reduces a symptom of the urogenital-neurological disorder, thereby treating the mammal.
91. A use of a modified Clostridial toxin for treating urogenital-neurological disorder in a mammal in need thereof, the use comprising the step of administering to the mammal in need thereof a therapeutically effective amount of a composition, wherein the modified Clostridial toxin comprising an opioid peptide binding domain, a Clostridial toxin translocation domain, a Clostridial toxin enzymatic domain, and an exogenous protease cleavage site, and wherein administration of the composition reduces a symptom of the urogenital-neurological disorder, thereby treating the mammal.

The following examples are provided by way of describing specific embodiments without intending to limit the scope of the invention in any way.

EXAMPLE 1

Treatment of Urinary Incontinence

A 69 year old female complains of the inability to control the passage of urine. A physician diagnosis the patient with urinary incontinence having a neurological component involving abnormal sensory neuron activity. The woman is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other

areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from treatment, and the woman indicates there is improvement of her ability to control the passage of urine. At one and three month check-ups, the woman indicates that she continues to have increased control over her ability to pass urine. This reduction in an urinary incontinence symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

A 72 year old female complains of the inability to control the passage of urine, and leakage occurs especially when she coughs, sneezes, laughs or exercises. A physician diagnosis the patient with stress urinary incontinence having a neurological component involving abnormal sensory neuron activity. The woman is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from treatment, and the woman indicates there is improvement of her ability to control the passage of urine, especially when she coughs, sneezes, laughs or exercises. At one and three month check-ups, the woman indicates that she continues to have increased control over her ability to pass urine. This reduction in a stress urinary incontinence symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

A 62 year old male complains of the inability to control the passage of urine, experiencing a sudden need to urinate. A physician diagnosis the patient with urge urinary incontinence having a neurological component involving abnormal sensory neuron activity. The man is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, lower pelvic muscles, prostate, bulbourethral gland, bulb, crus or penis. The patient's condition is monitored and after about 1-3 days from treatment, and the man indicates there is improvement of his ability to control the passage of urine because of a reduced sudden need to urinate. At one and three month check-ups, the man indicates that he continues to have increased control over his ability to pass urine. This reduction in an urge urinary incontinence symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

A 58 year old male complains of the inability to control the passage of urine because of leakage that occurs. A physician diagnosis the patient with overflow urinary incontinence having a neurological component involving abnormal sensory neuron activity that is causing blockage. The man is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, lower pelvic muscles, prostate, bulbourethral gland, bulb, crus or penis. The patient's condition is monitored and after about 1-3 days from treatment, and the man indicates there is improvement of his ability to control the passage of urine because of reduced leakage. At one and three month check-ups, the man indicates that he continues to have increased control over his ability to pass urine. This reduction in an overflow urinary incontinence symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

EXAMPLE 2

Treatment of Overactive Bladder

A 58 year old male complains of increased urinary urgency. A physician diagnosis the patient with overactive bladder having a neurological component involving abnormal sensory neuron activity. The man is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, lower pelvic muscles, prostate, bulbourethral gland, bulb, crus or penis. The patient's condition is monitored and after about 1-3 days from treatment, and the man indicates that he has a reduced urgency to urinate. At one and three month check-ups, the man indicates that he continues to have a reduced urgency to urinate. This reduction in an overactive bladder symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

A 66 year old female complains of having to wake up several times during the night to urinate. A physician determines that this is nocturia and diagnosis the patient with overactive bladder having a neurological component involving abnormal sensory neuron activity. The woman is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from treatment, and the woman indicates that she has a reduced need to wake up several times during the night to urinate. At one and three month check-ups, the woman indicates that she continues to have a reduced need to wake up several times during the night to urinate. This reduction in an overactive bladder symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

A 47 year old female complains of having to urinate several times a day. A physician determines that this is polyuria and diagnosis the patient with overactive bladder having a neurological component involving abnormal sensory neuron activity. The woman is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be admin-

istered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from treatment, and the woman indicates that she has a reduced need to urinate during the day. At one and three month check-ups, the woman indicates that she continues to have a reduced need urinate during the day. This reduction in an overactive bladder symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

A 67 year old male complains of the inability to control the passage of urine because of a sudden need to urinate. A physician determines that this is urge incontinence and diagnosis the patient with overactive bladder having a neurological component involving abnormal sensory neuron activity. The man is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, lower pelvic muscles, prostate, bulbourethral gland, bulb, crus or penis. The patient's condition is monitored and after about 1-3 days from treatment, and the man indicates that he has a reduced urgency to urinate. At one and three month check-ups, the man indicates that he continues to have a reduced urgency to urinate. This reduction in an overactive bladder symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

EXAMPLE 3

Treatment of Detrusor Dysfunction

A 44 year old female complains of uncontrollable bladder contractions. A physician determines that this is uninhibitable bladder contractions and diagnosis the patient with a detrusor dysfunction having a neurological component involving abnormal sensory neuron activity. The woman is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from treatment, and the woman indicates that there is a reduction in uncontrollable bladder contractions. At one and three month check-ups, the woman indicates that she continues to have a reduction in uncontrollable bladder contractions. This reduction in a detrusor dysfunction symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

In an alternative scenario, the physician determines that this is uninhibitable bladder contractions and diagnosis the patient with detrusor overactivity having a neurological component involving abnormal sensory neuron activity. The woman is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from treatment, and the woman indicates that there is a reduction in uncontrollable bladder contractions. At one and three month check-ups, the woman indicates that she continues to have a reduction in uncontrollable bladder contractions. This reduction in a detrusor overactivity symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

In another alternative scenario, the physician determines that this is uninhibitable bladder contractions and diagnosis the patient with detrusor instability having a neurological component involving abnormal sensory neuron activity. The woman is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from treatment, and the woman indicates that there is a reduction in uncontrollable bladder contractions. At one and three month check-ups, the woman indicates that she continues to have a reduction in uncontrollable bladder contractions. This reduction in a detrusor instability symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

A 50 year old female complains of an urgency to urinate. A physician determines that this is urinary urgency and diagnosis the patient with a detrusor dysfunction having a neurological component involving abnormal sensory neuron activity. The woman is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from treatment, and the woman indicates that there is a reduction in the urgency to urinate. At one and three month check-ups, the woman indicates that she continues to have a reduction in the urgency to urinate. This reduction in a detrusor dysfunction symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

In an alternative scenario, the physician determines that this is urinary urgency and diagnosis the patient with detrusor overactivity having a neurological component involving abnormal sensory neuron activity. The woman is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored

and after about 1-3 days from treatment, and the woman indicates that there is a reduction in the urgency to urinate. At one and three month check-ups, the woman indicates that she continues to have a reduction in the urgency to urinate. This reduction in a detrusor overactivity symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

In another alternative scenario, the physician determines that this is urinary urgency and diagnosis the patient with detrusor instability having a neurological component involving abnormal sensory neuron activity. The woman is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from treatment, and the woman indicates that there is a reduction in the urgency to urinate. At one and three month check-ups, the woman indicates that she continues to have a reduction in the urgency to urinate. This reduction in a detrusor instability symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

A 59 year old male complains of having to urinate all the time. A physician determines that this is urinary frequency and diagnosis the patient with a detrusor dysfunction having a neurological component involving abnormal sensory neuron activity. The man is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, lower pelvic muscles, prostate, bulbourethral gland, bulb, crus or penis. The patient's condition is monitored and after about 1-3 days from treatment, and the man indicates that there is a reduction in the need to urinate all the time. At one and three month check-ups, the man indicates that he continues to have a reduction in the need to urinate all the time. This reduction in a detrusor dysfunction symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

In an alternative scenario, the physician determines that this is urinary frequency and diagnosis the patient with detrusor overactivity having a neurological component involving abnormal sensory neuron activity. The man is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from treatment, and the man indicates that there is a reduction in the need to urinate all the time. At one and three month check-ups, the man indicates that he continues to have a reduction in the need to urinate all the time. This reduction in a detrusor overactivity symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

In another alternative scenario, the physician determines that this is urinary frequency and diagnosis the patient with detrusor instability having a neurological component involving abnormal sensory neuron activity. The man is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from treatment, and the man indicates that there is a reduction in the need to urinate all the time. At one and three month check-ups, the man indicates that he continues to have a reduction in the need to urinate all the time. This reduction in a detrusor instability symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

A 74 year old male complains of the involuntary loss of urine. A physician determines that this is enuresis and diagnosis the patient with a detrusor dysfunction having a neurological component involving abnormal sensory neuron activity. The man is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, lower pelvic muscles, prostate, bulbourethral gland, bulb, crus or penis. The patient's condition is monitored and after about 1-3 days from treatment, and the man indicates that there is a reduction in the involuntary loss of urine. At one and three month check-ups, the man indicates that he continues to have a reduction in the involuntary loss of urine. This reduction in a detrusor dysfunction symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

In an alternative scenario, the physician determines that this is enuresis and diagnosis the patient with detrusor overactivity having a neurological component involving abnormal sensory neuron activity. The man is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from treatment, and the man indicates that there is a reduction in the involuntary loss of urine. At one and three month check-ups, the man indicates that he continues to have a reduction in the involuntary loss of urine. This reduction in a detrusor overactivity symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

In another alternative scenario, the physician determines that this is enuresis and diagnosis the patient with detrusor instability having a neurological component involving abnormal sensory neuron activity. The man is treated by injecting

urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from treatment, and the man indicates that there is a reduction in the involuntary loss of urine. At one and three month check-ups, the man indicates that he continues to have a reduction in the involuntary loss of urine. This reduction in a detrusor instability symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

A 63 year old male complains of having to wake up several times during the night to urinate. A physician determines that this is nocturia and diagnosis the patient with a detrusor dysfunction having a neurological component involving abnormal sensory neuron activity. The man is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, lower pelvic muscles, prostate, bulbourethral gland, bulb, crus or penis. The patient's condition is monitored and after about 1-3 days from treatment, and the man indicates that there is a reduction in need to wake up several times during the night to urinate. At one and three month check-ups, the man indicates that he continues to have a reduction in need to wake up several times during the night to urinate. This reduction in a detrusor dysfunction symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

In an alternative scenario, the physician determines that this is nocturia and diagnosis the patient with detrusor overactivity having a neurological component involving abnormal sensory neuron activity. The man is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from treatment, and the man indicates that there is a reduction in need to wake up several times during the night to urinate. At one and three month check-ups, the man indicates that he continues to have a reduction in need to wake up several times during the night to urinate. This reduction in a detrusor overactivity symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

In another alternative scenario, the physician determines that this is nocturia and diagnosis the patient with detrusor instability having a neurological component involving abnormal sensory neuron activity. The man is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from treatment, and the man indicates that there is a reduction in need to wake up several times during the night to urinate. At one and three month check-ups, the man indicates that he continues to have a reduction in need to wake up several times during the night to urinate. This reduction in a detrusor overactivity symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

A 61 year old female complains of having to urinate several times a day. A physician determines that this is polyuria and diagnosis the patient with a detrusor dysfunction having a neurological component involving abnormal sensory neuron activity. The woman is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from treatment, and the woman indicates that there is a reduction in the need to urinate several times a day. At one and three month check-ups, the woman indicates that she continues to have a reduction in the need to urinate several times a day. This reduction in a detrusor dysfunction symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

In an alternative scenario, the physician determines that this is polyuria and diagnosis the patient with detrusor overactivity having a neurological component involving abnormal sensory neuron activity. The woman is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from treatment, and the woman indicates that there is a reduction in the need to urinate several times a day. At one and three month check-ups, the woman indicates that she continues to have a reduction in the need to urinate several times a day. This reduction in a detrusor overactivity symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

In another alternative scenario, the physician determines that this is polyuria and diagnosis the patient with detrusor instability having a neurological component involving abnormal sensory neuron activity. The woman is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the

bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from treatment, and the woman indicates that there is a reduction in the need to urinate several times a day. At one and three month check-ups, the woman indicates that she continues to have a reduction in the need to urinate several times a day. This reduction in a detrusor instability symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

A 65 year old female complains of the inability to control the passage of urine. A physician determines that this is urinary incontinence and diagnosis the patient with a detrusor dysfunction having a neurological component involving abnormal sensory neuron activity. The woman is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from the treatment, and the woman indicates there is improvement of her ability to control the passage of urine. At one and three month check-ups, the woman indicates that she continues to have an improved ability to control the passage of urine since the treatment. This reduction in a detrusor dysfunction symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

In an alternative scenario, the physician determines that this is urinary incontinence and diagnosis the patient with detrusor overactivity having a neurological component involving abnormal sensory neuron activity. The woman is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from the treatment, and the woman indicates there is improvement of her ability to control the passage of urine. At one and three month check-ups, the woman indicates that she continues to have an improved ability to control the passage of urine since the treatment. This reduction in a detrusor overactivity symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

In another alternative scenario, the physician determines that this is urinary incontinence and diagnosis the patient with detrusor instability having a neurological component involving abnormal sensory neuron activity. The woman is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from the treatment, and the woman indicates there is improvement of her ability to control the passage of urine. At one and three month check-ups, the woman indicates that she continues to have an improved ability to control the passage of urine since the treatment. This reduction in a detrusor instability symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

A 55 year old female complains of an interruption of urine flow when she urinates. A physician diagnosis the patient with a detrusor dysfunction having a neurological component involving abnormal sensory neuron activity. The woman is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from treatment, and the woman indicates that there is a reduction in urine flow interruption. At one and three month check-ups, the woman indicates that she continues to have a reduced urine flow interruption since the treatment. This reduction in a detrusor dysfunction symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

In an alternative scenario, the physician diagnosis the patient with a detrusor-sphincter dyssynergia having a neurological component involving abnormal sensory neuron activity. The woman is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from treatment, and the woman indicates that there is a reduction in urine flow interruption. At one and three month check-ups, the woman indicates that she continues to have a reduced urine flow interruption since the treatment. This reduction in a detrusor-sphincter dyssynergia symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

A 53 year old male complains of increased bladder pressure. A physician determines that this is raised detrusor pressure and diagnosis the patient with a detrusor dysfunction having a neurological component involving abnormal sensory neuron activity. The man is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, lower pelvic muscles, prostate, bulbourethral gland, bulb, crus or penis. The patient's condition is monitored and after about 1-3 days from treatment, and the man indicates that there is a reduction in bladder pressure. At one and three month check-ups, the man indicates that he continues to have a reduced bladder pressure

since the treatment. This reduction in a detrusor dysfunction symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

In an alternative scenario, the physician determines that this is raised detrusor pressure and diagnosis the patient with a detrusor-sphincter dyssynergia having a neurological component involving abnormal sensory neuron activity. The man is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from treatment, and the man indicates that there is a reduction in bladder pressure. At one and three month check-ups, the man indicates that he continues to have a reduced bladder pressure since the treatment. This reduction in a detrusor-sphincter dyssynergia symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

A 75 year old male complains of the inability to urinate. A physician determines that this is urinary retention and diagnosis the patient with a detrusor dysfunction having a neurological component involving abnormal sensory neuron activity. The man is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, lower pelvic muscles, prostate, bulbourethral gland, bulb, crus or penis. The patient's condition is monitored and after about 1-3 days from treatment, and the man indicates that he has regained the ability to urinate. At one and three month check-ups, the man indicates that he continues to have the ability to urinate. This reduction in a detrusor dysfunction symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

In an alternative scenario, the physician determines that this is urinary retention and diagnosis the patient with a detrusor-sphincter dyssynergia having a neurological component involving abnormal sensory neuron activity. The man is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from treatment, and the man indicates that he has regained the ability to urinate. At one and three month check-ups, the man indicates that he continues to have the ability to urinate. This reduction in a detrusor-sphincter dyssynergia symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

EXAMPLE 4

Treatment of Lower Urinary Tract Dysfunction

A 69 year old male complains of the need to urinate suddenly. A physician determines that this is a urine storage problem and diagnosis the patient with a lower urinary tract dysfunction having a neurological component involving abnormal sensory neuron activity. The man is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, lower pelvic muscles, prostate, bulbourethral gland, bulb, crus or penis. The patient's condition is monitored and after about 1-3 days from treatment, and the man indicates that there is a reduction in the sudden need to urinate. At one and three month check-ups, the man indicates that he still experiences a reduced need to urinate. This reduction in a lower urinary tract dysfunction indicates successful treatment with the composition comprising a modified Clostridial toxin. In similar scenarios the patient could have complained of other storage symptoms of lower urinary tract dysfunction such as, e.g., urinary frequency, enuresis, polyuria, nocturia increased bladder sensation, decreased bladder sensation, absent bladder sensation, non-specific bladder sensation, and/or urinary incontinence. In each case, after diagnosis of lower urinary tract dysfunction, a physician would treat the patient as indicated above and there would be a reduction in the lower urinary tract dysfunction storage symptom.

A 70 year old male complains of having difficulty urinating and having to strain in order to urinate. A physician determines that this is a urine voiding problem and diagnosis the patient with a lower urinary tract dysfunction having a neurological component involving abnormal sensory neuron activity. The man is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, lower pelvic muscles, prostate, bulbourethral gland, bulb, crus or penis. The patient's condition is monitored and after about 1-3 days from treatment, and the man indicates that it is easier to urinate and he does not have to strain as much in order to urinate. At one and three month check-ups, the man indicates that he still experiences an easier time to urinate. This reduction in a lower urinary tract dysfunction indicates successful treatment with the composition comprising a modified Clostridial toxin. In similar scenarios the patient could have complained of other voiding symptoms of lower urinary tract dysfunction such as, e.g., reduced urine flow, splitting or spraying of urine, intermittent urine flow, urinary hesitancy, and/or terminal dribble of urine. In each case, after diagnosis of lower urinary tract dysfunction, a physician would treat the patient as indicated above and there would be a reduction in the lower urinary tract dysfunction voiding symptom.

A 77 year old male complains of urine dribbling after he finishes urinating. A physician determines that this is a urine post-micturition problem and diagnosis the patient with a lower urinary tract dysfunction having a neurological component involving abnormal sensory neuron activity. The man is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the

detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, lower pelvic muscles, prostate, bulbourethral gland, bulb, crus or penis. The patient's condition is monitored and after about 1-3 days from treatment, and the man indicates that there is a reduction in urine dribbling after he finishes urinating. At one and three month check-ups, the man indicates that he still experiences reduced dribbling after he finishes urinating. This reduction in a lower urinary tract dysfunction indicates successful treatment with the composition comprising a modified Clostridial toxin. In similar scenarios the patient could have complained of other post-micturition symptoms of lower urinary tract dysfunction such as, e.g., sensation of incomplete emptying. In each case, after diagnosis of lower urinary tract dysfunction, a physician would treat the patient as indicated above and there would be a reduction in the lower urinary tract dysfunction post-micturition symptom.

EXAMPLE 5

Treatment of Urinary Retention

A 79 year old female complains that she cannot urinate. A physician diagnosis the patient with urinary retention having a neurological component involving abnormal sensory neuron activity. The woman is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from treatment, and the woman indicates that she has regained the ability to urinate. At one and three month check-ups, the woman indicates that she still continues to have control over her ability to urinate. This reduction in a urinary retention symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

EXAMPLE 6

Treatment of Urinary Hesitancy

A 78 year old male complains that he has difficulty starting and/or maintaining his ability to urinate. A physician diagnosis the patient with urinary hesitancy having a neurological component involving abnormal sensory neuron activity. The man is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, lower pelvic muscles, prostate, bulbourethral gland, bulb, crus or penis. The patient's condition is monitored and after about 1-3 days from treatment, and the man indicates that he has less difficulty in starting and/or maintaining his ability to urinate. At one and three month check-ups, the man indicates that he still experiences less difficulty in starting and/or maintaining his ability to urinate. This reduction in a urinary hesitancy symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

EXAMPLE 7

Treatment of Polyuria

A 68 year old male complains that he has to urinate all the time during the day. A physician diagnosis the patient with polyuria having a neurological component involving abnormal sensory neuron activity. The man is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, lower pelvic muscles, prostate, bulbourethral gland, bulb, crus or penis. The patient's condition is monitored and after about 1-3 days from treatment, and the man indicates that does not have to urinate as many times during the day as before the treatment. At one and three month check-ups, the man still indicates that does not have to urinate as many times during the day as before the treatment. This reduction in a polyuria symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

EXAMPLE 8

Treatment of Nocturia

A 57 year old female complains that she has to wake up several times during the night in order to urinate. A physician diagnosis the patient with nocturia having a neurological component involving abnormal sensory neuron activity. The woman is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from treatment, and the woman indicates that she does not have to get up as many times during the night to urinate as she did before the treatment. At one and three month check-ups, the woman still indicates that she does not have to get up as many times during the night to urinate as she did before the treatment. This reduction in a nocturia symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

EXAMPLE 9

Treatment of Chronic Urinary Tract Infection

A 76 year old female complains that she has urinary tract infections all the time. A physician determines that the chronic urinary tract infections is abacterial and diagnosis the patient with urogential disorder having a neurological component involving abnormal sensory neuron activity. The woman is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in

the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from treatment, and the physician indicates that she does not have a urinary tract infection. At one and three month check-ups, the woman indicates that she has not had a urinary tract infection since the treatment. This reduction in a urinary tract infection symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

A 75 year old female complains that she has urinary tract infections all the time. A physician determines that the chronic urinary tract infection is due to vesicoureteral reflux and diagnosis the patient with urogential disorder having a neurological component involving abnormal sensory neuron activity. The woman is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from treatment, and the physician determines that the abnormal backup of urine from the bladder to the kidneys is reduced in the patient. At one and three month check-ups, the woman indicates that she has not had a urinary tract infection since the treatment. This reduction in a urinary tract infection symptom indicates successful treatment with the composition comprising a modified Clostridial toxin.

EXAMPLE 10

Treatment of Urogenital Disorder Associated with a Prostate Disorder

A 78 year old male complains that he has difficulty starting and/or maintaining his ability to urinate. A physician determines that he has benign prostatic hyperplasia and that this enlargement is blocking the normal flow of urine. The physician diagnosis the patient with urinary hesitancy associated with benign prostatic hyperplasia having a neurological component involving abnormal sensory neuron activity. The man is treated by injecting a composition comprising a modified Clostridial toxin as disclosed in the present specification into the prostate and/or in the surrounding area of the prostate depending on the location of abnormal sensory neuron activity. The patient's condition is monitored and after about 1-2 weeks from the treatment, the man indicates that he has less difficulty in starting and/or maintaining his ability to urinate. The physician determines that the size of the prostate has reduced since the treatment. At one and three month check-ups, the man indicates that he still experiences less difficulty in starting and/or maintaining his ability to urinate. This reduction in a urinary hesitancy symptom associated with benign prostatic hyperplasia indicates successful treatment with the composition comprising a modified Clostridial toxin.

EXAMPLE 11

Treatment of Urogenital Disorder Associated with a Neurogenic Dysfunction

A 81 year old female diagnosed with Parkinson's Disease complains about having a sudden need to urinate. A physician determines that this urinary urgency is due to her Parkinson's Disease and diagnosis the patient with urogential disorder associated with a neurogenic dysfunction having a neurological component involving abnormal sensory neuron activity. The woman is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from treatment, and the woman indicates that there is a reduction in the sudden need to urinate. At one and three month check-ups, the woman indicates that she continues to experience a reduced sudden need to urinate. This reduction in a urogenital disorder symptom associated with a neurogenic dysfunction indicates successful treatment with the composition comprising a modified Clostridial toxin.

A 39 year old female diagnosed with multiple sclerosis complains about having a need to urinate all the time. A physician determines that this urinary frequency is due to her multiple sclerosis and diagnosis the patient with urogential disorder associated with a neurogenic dysfunction having a neurological component involving abnormal sensory neuron activity. The woman is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, or lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from treatment, and the woman indicates that there is a reduction in the need to urinate all the time. At one and three month check-ups, the woman indicates that she still experiences a reduced need to urinate all the time. This reduction in a urogenital disorder symptom associated with a neurogenic dysfunction indicates successful treatment with the composition comprising a modified Clostridial toxin.

A 12 year old male diagnosed with spina bifida complains about the inability to control the passage of urine. A physician determines that this urinary incontinence is due to his spina bifida and diagnosis the patient with urogential disorder associated with a neurogenic dysfunction having a neurological component involving abnormal sensory neuron activity. The boy is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, lower pelvic muscles, prostate, bulbourethral gland, bulb, crus or penis. The patient's condition is monitored and after

about 1-3 days from treatment, and the boy indicates that he has an increased ability to control the passage or urine. At one and three month check-ups, the boy indicates that he still experiences an increased ability to control the passage or urine. This reduction in a urogenital disorder symptom associated with a neurogenic dysfunction indicates successful treatment with the composition comprising a modified Clostridial toxin.

A 84 year old male who experienced a stroke complains about not being able to urinate. A physician determines that this urinary retention is due to his stroke and diagnosis the patient with urogential disorder associated with a neurogenic dysfunction having a neurological component involving abnormal sensory neuron activity. The man is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, lower pelvic muscles, prostate, bulbourethral gland, bulb, crus or penis. The patient's condition is monitored and after about 1-3 days from treatment, and the man indicates that he can urinate. At one and three month check-ups, the man indicates that he continues to experience the ability to urinate. This reduction in a urogenital disorder symptom associated with a neurogenic dysfunction indicates successful treatment with the composition comprising a modified Clostridial toxin.

A 23 year old man suffering from a spinal cord injury resulting from a car accident complains about the inability to control the passage of urine. A physician determines that this urinary incontinence is due to his spinal cord injury and diagnosis the patient with urogential disorder associated with a neurogenic dysfunction having a neurological component involving abnormal sensory neuron activity. The man is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, lower pelvic muscles. The patient's condition is monitored and after about 1-3 days from treatment, and the man indicates that he has an increased ability to control the passage or urine. At one and three month check-ups, the man indicates that he still experiences an increased ability to control the passage or urine. This reduction in a urogenital disorder symptom associated with a neurogenic dysfunction indicates successful treatment with the composition comprising a modified Clostridial toxin.

A 63 year old male who has cancerous lesion in his brain complains about having a need to urinate all the time. A physician determines that this urinary frequency is due to his lesion and diagnosis the patient with urogential disorder associated with a neurogenic dysfunction having a neurological component involving abnormal sensory neuron activity. The man is treated by injecting urethroscopically a composition comprising a modified Clostridial toxin as disclosed in the present specification. Depending on the location of abnormal sensory neuron activity, the toxin can be administered into e.g., the detrusor, the bladder neck including the external and internal urethral sphincters, the trigone, the bladder dome or other areas of the bladder wall, and/or other areas surrounding the bladder, such as the urethra, ureter, urogenital diaphragm, lower pelvic muscles, prostate, bulbourethral gland, bulb, crus or penis. The patient's condition is monitored and after about 1-3 days from treatment, and the man indicates that there is a reduction in the need to urinate all the time. At one and three month check-ups, the man indicates that he still experiences a reduced need to urinate all the time. This reduction in a urogenital disorder symptom associated with a neurogenic dysfunction indicates successful treatment with the composition comprising a modified Clostridial toxin.

The foregoing description of the invention is exemplary for purposes of illustration and explanation. It will be apparent to those skilled in the art that changes and modifications are possible without departing from the spirit and scope of the invention. All documents cited herein are hereby incorporated by reference. It is intended that the following claims be interpreted to embrace all such changes and modifications.

SEQUENCE LISTING

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<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum Serotype A
<220> FEATURE:
<221> NAME/KEY: DOMAIN
<222> LOCATION: (1)...(448)
<223> OTHER INFORMATION: Light chain comprising the enzymatic domain.
<221> NAME/KEY: DOMAIN
<222> LOCATION: (449)...(860)
<223> OTHER INFORMATION: Amino-terminal half of heavy chain comprising the translocation domain.
<221> NAME/KEY: DOMAIN
<222> LOCATION: (861)...(1296)
<223> OTHER INFORMATION: Carboxyl-terminal half of heavy chain comprising the binding domain.

<400> SEQUENCE: 1

Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly
1 5 10 15

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Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
            20                  25                  30

Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
        35                  40                  45

Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu
    50                  55                  60

Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr
65                  70                  75                  80

Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu
                85                  90                  95

Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val
            100                 105                 110

Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys
        115                 120                 125

Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr
    130                 135                 140

Arg Ser Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile
145                 150                 155                 160

Ile Gln Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr
                165                 170                 175

Arg Asn Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe
            180                 185                 190

Thr Phe Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu
        195                 200                 205

Gly Ala Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu
    210                 215                 220

Leu Ile His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile Asn Pro Asn
225                 230                 235                 240

Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ser Gly Leu
                245                 250                 255

Glu Val Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His Asp Ala Lys
            260                 265                 270

Phe Ile Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn
        275                 280                 285

Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val
    290                 295                 300

Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys
305                 310                 315                 320

Tyr Leu Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val Asp Lys Leu
                325                 330                 335

Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp
            340                 345                 350

Asn Phe Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr Tyr Leu Asn
        355                 360                 365

Phe Asp Lys Ala Val Phe Lys Ile Asn Ile Val Pro Lys Val Asn Tyr
    370                 375                 380

Thr Ile Tyr Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn
385                 390                 395                 400

Phe Asn Gly Gln Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu
                405                 410                 415

Lys Asn Phe Thr Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg
            420                 425                 430

Gly Ile Ile Thr Ser Lys Thr Lys Ser Leu Asp Lys Gly Tyr Asn Lys
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        435                 440                 445

Ala Leu Asn Asp Leu Cys Ile Lys Val Asn Asn Trp Asp Leu Phe Phe
    450                 455                 460

Ser Pro Ser Glu Asp Asn Phe Thr Asn Asp Leu Asn Lys Gly Glu Glu
465                 470                 475                 480

Ile Thr Ser Asp Thr Asn Ile Glu Ala Ala Glu Glu Asn Ile Ser Leu
                485                 490                 495

Asp Leu Ile Gln Gln Tyr Tyr Leu Thr Phe Asn Phe Asp Asn Glu Pro
            500                 505                 510

Glu Asn Ile Ser Ile Glu Asn Leu Ser Ser Asp Ile Ile Gly Gln Leu
        515                 520                 525

Glu Leu Met Pro Asn Ile Glu Arg Phe Pro Asn Gly Lys Lys Tyr Glu
    530                 535                 540

Leu Asp Lys Tyr Thr Met Phe His Tyr Leu Arg Ala Gln Glu Phe Glu
545                 550                 555                 560

His Gly Lys Ser Arg Ile Ala Leu Thr Asn Ser Val Asn Glu Ala Leu
                565                 570                 575

Leu Asn Pro Ser Arg Val Tyr Thr Phe Phe Ser Ser Asp Tyr Val Lys
            580                 585                 590

Lys Val Asn Lys Ala Thr Glu Ala Ala Met Phe Leu Gly Trp Val Glu
        595                 600                 605

Gln Leu Val Tyr Asp Phe Thr Asp Glu Thr Ser Glu Val Ser Thr Thr
    610                 615                 620

Asp Lys Ile Ala Asp Ile Thr Ile Ile Ile Pro Tyr Ile Gly Pro Ala
625                 630                 635                 640

Leu Asn Ile Gly Asn Met Leu Tyr Lys Asp Asp Phe Val Gly Ala Leu
                645                 650                 655

Ile Phe Ser Gly Ala Val Ile Leu Leu Glu Phe Ile Pro Glu Ile Ala
            660                 665                 670

Ile Pro Val Leu Gly Thr Phe Ala Leu Val Ser Tyr Ile Ala Asn Lys
        675                 680                 685

Val Leu Thr Val Gln Thr Ile Asp Asn Ala Leu Ser Lys Arg Asn Glu
    690                 695                 700

Lys Trp Asp Glu Val Tyr Lys Tyr Ile Val Thr Asn Trp Leu Ala Lys
705                 710                 715                 720

Val Asn Thr Gln Ile Asp Leu Ile Arg Lys Lys Met Lys Glu Ala Leu
                725                 730                 735

Glu Asn Gln Ala Glu Ala Thr Lys Ala Ile Ile Asn Tyr Gln Tyr Asn
            740                 745                 750

Gln Tyr Thr Glu Glu Glu Lys Asn Asn Ile Asn Phe Asn Ile Asp Asp
        755                 760                 765

Leu Ser Ser Lys Leu Asn Glu Ser Ile Asn Lys Ala Met Ile Asn Ile
    770                 775                 780

Asn Lys Phe Leu Asn Gln Cys Ser Val Ser Tyr Leu Met Asn Ser Met
785                 790                 795                 800

Ile Pro Tyr Gly Val Lys Arg Leu Glu Asp Phe Asp Ala Ser Leu Lys
                805                 810                 815

Asp Ala Leu Leu Lys Tyr Ile Tyr Asp Asn Arg Gly Thr Leu Ile Gly
            820                 825                 830

Gln Val Asp Arg Leu Lys Asp Lys Val Asn Asn Thr Leu Ser Thr Asp
        835                 840                 845

Ile Pro Phe Gln Leu Ser Lys Tyr Val Asp Asn Gln Arg Leu Leu Ser
    850                 855                 860
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Thr Phe Thr Glu Tyr Ile Lys Asn Ile Ile Asn Thr Ser Ile Leu Asn
865                 870                 875                 880

Leu Arg Tyr Glu Ser Asn His Leu Ile Asp Leu Ser Arg Tyr Ala Ser
                885                 890                 895

Lys Ile Asn Ile Gly Ser Lys Val Asn Phe Asp Pro Ile Asp Lys Asn
            900                 905                 910

Gln Ile Gln Leu Phe Asn Leu Glu Ser Ser Lys Ile Glu Val Ile Leu
        915                 920                 925

Lys Asn Ala Ile Val Tyr Asn Ser Met Tyr Glu Asn Phe Ser Thr Ser
    930                 935                 940

Phe Trp Ile Arg Ile Pro Lys Tyr Phe Asn Ser Ile Ser Leu Asn Asn
945                 950                 955                 960

Glu Tyr Thr Ile Ile Asn Cys Met Glu Asn Asn Ser Gly Trp Lys Val
                965                 970                 975

Ser Leu Asn Tyr Gly Glu Ile Ile Trp Thr Leu Gln Asp Thr Gln Glu
            980                 985                 990

Ile Lys Gln Arg Val Val Phe Lys Tyr Ser Gln Met Ile Asn Ile Ser
        995                 1000                1005

Asp Tyr Ile Asn Arg Trp Ile Phe Val Thr Ile Thr Asn Asn Arg Leu
    1010                1015                1020

Asn Asn Ser Lys Ile Tyr Ile Asn Gly Arg Leu Ile Asp Gln Lys Pro
1025                1030                1035                1040

Ile Ser Asn Leu Gly Asn Ile His Ala Ser Asn Asn Ile Met Phe Lys
                1045                1050                1055

Leu Asp Gly Cys Arg Asp Thr His Arg Tyr Ile Trp Ile Lys Tyr Phe
            1060                1065                1070

Asn Leu Phe Asp Lys Glu Leu Asn Glu Lys Glu Ile Lys Asp Leu Tyr
        1075                1080                1085

Asp Asn Gln Ser Asn Ser Gly Ile Leu Lys Asp Phe Trp Gly Asp Tyr
    1090                1095                1100

Leu Gln Tyr Asp Lys Pro Tyr Tyr Met Leu Asn Leu Tyr Asp Pro Asn
1105                1110                1115                1120

Lys Tyr Val Asp Val Asn Asn Val Gly Ile Arg Gly Tyr Met Tyr Leu
                1125                1130                1135

Lys Gly Pro Arg Gly Ser Val Met Thr Thr Asn Ile Tyr Leu Asn Ser
            1140                1145                1150

Ser Leu Tyr Arg Gly Thr Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly
        1155                1160                1165

Asn Lys Asp Asn Ile Val Arg Asn Asn Asp Arg Val Tyr Ile Asn Val
    1170                1175                1180

Val Val Lys Asn Lys Glu Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala
1185                1190                1195                1200

Gly Val Glu Lys Ile Leu Ser Ala Leu Glu Ile Pro Asp Val Gly Asn
                1205                1210                1215

Leu Ser Gln Val Val Val Met Lys Ser Lys Asn Asp Gln Gly Ile Thr
            1220                1225                1230

Asn Lys Cys Lys Met Asn Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly
        1235                1240                1245

Phe Ile Gly Phe His Gln Phe Asn Asn Ile Ala Lys Leu Val Ala Ser
    1250                1255                1260

Asn Trp Tyr Asn Arg Gln Ile Glu Arg Ser Ser Arg Thr Leu Gly Cys
1265                1270                1275                1280
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Ser Trp Glu Phe Ile Pro Val Asp Asp Gly Trp Gly Glu Arg Pro Leu
1285 1290 1295

<210> SEQ ID NO 2
<211> LENGTH: 1291
<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum Serotype B
<220> FEATURE:
<221> NAME/KEY: DOMAIN
<222> LOCATION: (1)...(441)
<223> OTHER INFORMATION: Light chain comprising the enzymatic domain.
<221> NAME/KEY: DOMAIN
<222> LOCATION: (442)...(847)
<223> OTHER INFORMATION: Amino-terminal half of heavy chain comprising the translocation domain.
<221> NAME/KEY: DOMAIN
<222> LOCATION: (848)...(1291)
<223> OTHER INFORMATION: Carboxyl-terminal half of heavy chain comprising the binding domain.

<400> SEQUENCE: 2

Met Pro Val Thr Ile Asn Asn Phe Asn Tyr Asn Asp Pro Ile Asp Asn
1 5 10 15

Asn Asn Ile Ile Met Met Glu Pro Pro Phe Ala Arg Gly Thr Gly Arg
20 25 30

Tyr Tyr Lys Ala Phe Lys Ile Thr Asp Arg Ile Trp Ile Ile Pro Glu
35 40 45

Arg Tyr Thr Phe Gly Tyr Lys Pro Glu Asp Phe Asn Lys Ser Ser Gly
50 55 60

Ile Phe Asn Arg Asp Val Cys Glu Tyr Tyr Asp Pro Asp Tyr Leu Asn
65 70 75 80

Thr Asn Asp Lys Lys Asn Ile Phe Leu Gln Thr Met Ile Lys Leu Phe
85 90 95

Asn Arg Ile Lys Ser Lys Pro Leu Gly Glu Lys Leu Leu Glu Met Ile
100 105 110

Ile Asn Gly Ile Pro Tyr Leu Gly Asp Arg Arg Val Pro Leu Glu Glu
115 120 125

Phe Asn Thr Asn Ile Ala Ser Val Thr Val Asn Lys Leu Ile Ser Asn
130 135 140

Pro Gly Glu Val Glu Arg Lys Lys Gly Ile Phe Ala Asn Leu Ile Ile
145 150 155 160

Phe Gly Pro Gly Pro Val Leu Asn Glu Asn Glu Thr Ile Asp Ile Gly
165 170 175

Ile Gln Asn His Phe Ala Ser Arg Glu Gly Phe Gly Gly Ile Met Gln
180 185 190

Met Lys Phe Cys Pro Glu Tyr Val Ser Val Phe Asn Asn Val Gln Glu
195 200 205

Asn Lys Gly Ala Ser Ile Phe Asn Arg Arg Gly Tyr Phe Ser Asp Pro
210 215 220

Ala Leu Ile Leu Met His Glu Leu Ile His Val Leu His Gly Leu Tyr
225 230 235 240

Gly Ile Lys Val Asp Asp Leu Pro Ile Val Pro Asn Glu Lys Lys Phe
245 250 255

Phe Met Gln Ser Thr Asp Ala Ile Gln Ala Glu Glu Leu Tyr Thr Phe
260 265 270

Gly Gly Gln Asp Pro Ser Ile Ile Thr Pro Ser Thr Asp Lys Ser Ile
275 280 285

Tyr Asp Lys Val Leu Gln Asn Phe Arg Gly Ile Val Asp Arg Leu Asn
290 295 300

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Lys Val Leu Val Cys Ile Ser Asp Pro Asn Ile Asn Ile Asn Ile Tyr
305                 310                 315                 320

Lys Asn Lys Phe Lys Asp Lys Tyr Lys Phe Val Glu Asp Ser Glu Gly
                325                 330                 335

Lys Tyr Ser Ile Asp Val Glu Ser Phe Asp Lys Leu Tyr Lys Ser Leu
            340                 345                 350

Met Phe Gly Phe Thr Glu Thr Asn Ile Ala Glu Asn Tyr Lys Ile Lys
        355                 360                 365

Thr Arg Ala Ser Tyr Phe Ser Asp Ser Leu Pro Pro Val Lys Ile Lys
    370                 375                 380

Asn Leu Leu Asp Asn Glu Ile Tyr Thr Ile Glu Glu Gly Phe Asn Ile
385                 390                 395                 400

Ser Asp Lys Asp Met Glu Lys Glu Tyr Arg Gly Gln Asn Lys Ala Ile
                405                 410                 415

Asn Lys Gln Ala Tyr Glu Glu Ile Ser Lys Glu His Leu Ala Val Tyr
            420                 425                 430

Lys Ile Gln Met Cys Lys Ser Val Lys Ala Pro Gly Ile Cys Ile Asp
        435                 440                 445

Val Asp Asn Glu Asp Leu Phe Phe Ile Ala Asp Lys Asn Ser Phe Ser
    450                 455                 460

Asp Asp Leu Ser Lys Asn Glu Arg Ile Glu Tyr Asn Thr Gln Ser Asn
465                 470                 475                 480

Tyr Ile Glu Asn Asp Phe Pro Ile Asn Glu Leu Ile Leu Asp Thr Asp
                485                 490                 495

Leu Ile Ser Lys Ile Glu Leu Pro Ser Glu Asn Thr Glu Ser Leu Thr
            500                 505                 510

Asp Phe Asn Val Asp Val Pro Val Tyr Glu Lys Gln Pro Ala Ile Lys
        515                 520                 525

Lys Ile Phe Thr Asp Glu Asn Thr Ile Phe Gln Tyr Leu Tyr Ser Gln
    530                 535                 540

Thr Phe Pro Leu Asp Ile Arg Asp Ile Ser Leu Thr Ser Ser Phe Asp
545                 550                 555                 560

Asp Ala Leu Leu Phe Ser Asn Lys Val Tyr Ser Phe Phe Ser Met Asp
                565                 570                 575

Tyr Ile Lys Thr Ala Asn Lys Val Val Glu Ala Gly Leu Phe Ala Gly
            580                 585                 590

Trp Val Lys Gln Ile Val Asn Asp Phe Val Ile Glu Ala Asn Lys Ser
        595                 600                 605

Asn Thr Met Asp Lys Ile Ala Asp Ile Ser Leu Ile Val Pro Tyr Ile
    610                 615                 620

Gly Leu Ala Leu Asn Val Gly Asn Glu Thr Ala Lys Gly Asn Phe Glu
625                 630                 635                 640

Asn Ala Phe Glu Ile Ala Gly Ala Ser Ile Leu Leu Glu Phe Ile Pro
                645                 650                 655

Glu Leu Leu Ile Pro Val Val Gly Ala Phe Leu Leu Glu Ser Tyr Ile
            660                 665                 670

Asp Asn Lys Asn Lys Ile Ile Lys Thr Ile Asp Asn Ala Leu Thr Lys
        675                 680                 685

Arg Asn Glu Lys Trp Ser Asp Met Tyr Gly Leu Ile Val Ala Gln Trp
    690                 695                 700

Leu Ser Thr Val Asn Thr Gln Phe Tyr Thr Ile Lys Glu Gly Met Tyr
705                 710                 715                 720
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Lys Ala Leu Asn Tyr Gln Ala Gln Ala Leu Glu Glu Ile Ile Lys Tyr
                725                 730                 735

Arg Tyr Asn Ile Tyr Ser Glu Lys Glu Lys Ser Asn Ile Asn Ile Asp
            740                 745                 750

Phe Asn Asp Ile Asn Ser Lys Leu Asn Glu Gly Ile Asn Gln Ala Ile
        755                 760                 765

Asp Asn Ile Asn Asn Phe Ile Asn Gly Cys Ser Val Ser Tyr Leu Met
    770                 775                 780

Lys Lys Met Ile Pro Leu Ala Val Glu Lys Leu Leu Asp Phe Asp Asn
785                 790                 795                 800

Thr Leu Lys Lys Asn Leu Leu Asn Tyr Ile Asp Glu Asn Lys Leu Tyr
                805                 810                 815

Leu Ile Gly Ser Ala Glu Tyr Glu Lys Ser Lys Val Asn Lys Tyr Leu
            820                 825                 830

Lys Thr Ile Met Pro Phe Asp Leu Ser Ile Tyr Thr Asn Asp Thr Ile
        835                 840                 845

Leu Ile Glu Met Phe Asn Lys Tyr Asn Ser Glu Ile Leu Asn Asn Ile
    850                 855                 860

Ile Leu Asn Leu Arg Tyr Lys Asp Asn Asn Leu Ile Asp Leu Ser Gly
865                 870                 875                 880

Tyr Gly Ala Lys Val Glu Val Tyr Asp Gly Val Glu Leu Asn Asp Lys
                885                 890                 895

Asn Gln Phe Lys Leu Thr Ser Ser Ala Asn Ser Lys Ile Arg Val Thr
            900                 905                 910

Gln Asn Gln Asn Ile Ile Phe Asn Ser Val Phe Leu Asp Phe Ser Val
        915                 920                 925

Ser Phe Trp Ile Arg Ile Pro Lys Tyr Lys Asn Asp Gly Ile Gln Asn
    930                 935                 940

Tyr Ile His Asn Glu Tyr Thr Ile Ile Asn Cys Met Lys Asn Asn Ser
945                 950                 955                 960

Gly Trp Lys Ile Ser Ile Arg Gly Asn Arg Ile Ile Trp Thr Leu Ile
                965                 970                 975

Asp Ile Asn Gly Lys Thr Lys Ser Val Phe Phe Glu Tyr Asn Ile Arg
            980                 985                 990

Glu Asp Ile Ser Glu Tyr Ile Asn Arg Trp Phe Phe Val Thr Ile Thr
        995                 1000                1005

Asn Asn Leu Asn Asn Ala Lys Ile Tyr Ile Asn Gly Lys Leu Glu Ser
    1010                1015                1020

Asn Thr Asp Ile Lys Asp Ile Arg Glu Val Ile Ala Asn Gly Glu Ile
1025                1030                1035                1040

Ile Phe Lys Leu Asp Gly Asp Ile Asp Arg Thr Gln Phe Ile Trp Met
                1045                1050                1055

Lys Tyr Phe Ser Ile Phe Asn Thr Glu Leu Ser Gln Ser Asn Ile Glu
            1060                1065                1070

Glu Arg Tyr Lys Ile Gln Ser Tyr Ser Glu Tyr Leu Lys Asp Phe Trp
        1075                1080                1085

Gly Asn Pro Leu Met Tyr Asn Lys Glu Tyr Tyr Met Phe Asn Ala Gly
    1090                1095                1100

Asn Lys Asn Ser Tyr Ile Lys Leu Lys Lys Asp Ser Pro Val Gly Glu
1105                1110                1115                1120

Ile Leu Thr Arg Ser Lys Tyr Asn Gln Asn Ser Lys Tyr Ile Asn Tyr
                1125                1130                1135

Arg Asp Leu Tyr Ile Gly Glu Lys Phe Ile Ile Arg Arg Lys Ser Asn
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            1140                1145                1150

Ser Gln Ser Ile Asn Asp Asp Ile Val Arg Lys Glu Asp Tyr Ile Tyr
        1155                1160                1165

Leu Asp Phe Phe Asn Leu Asn Gln Glu Trp Arg Val Tyr Thr Tyr Lys
    1170                1175                1180

Tyr Phe Lys Lys Glu Glu Glu Lys Leu Phe Leu Ala Pro Ile Ser Asp
1185                1190                1195                1200

Ser Asp Glu Phe Tyr Asn Thr Ile Gln Ile Lys Glu Tyr Asp Glu Gln
                1205                1210                1215

Pro Thr Tyr Ser Cys Gln Leu Leu Phe Lys Lys Asp Glu Glu Ser Thr
            1220                1225                1230

Asp Glu Ile Gly Leu Ile Gly Ile His Arg Phe Tyr Glu Ser Gly Ile
        1235                1240                1245

Val Phe Glu Glu Tyr Lys Asp Tyr Phe Cys Ile Ser Lys Trp Tyr Leu
    1250                1255                1260

Lys Glu Val Lys Arg Lys Pro Tyr Asn Leu Lys Leu Gly Cys Asn Trp
1265                1270                1275                1280

Gln Phe Ile Pro Lys Asp Glu Gly Trp Thr Glu
                1285                1290


<210> SEQ ID NO 3
<211> LENGTH: 1291
<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum Serotype C1
<220> FEATURE:
<221> NAME/KEY: DOMAIN
<222> LOCATION: (1)...(449)
<223> OTHER INFORMATION: Light chain comprising the enzymatic domain.
<221> NAME/KEY: DOMAIN
<222> LOCATION: (450)...(855)
<223> OTHER INFORMATION: Amino-terminal half of heavy chain comprising
      the translocation domain.
<221> NAME/KEY: DOMAIN
<222> LOCATION: (856)...(1291)
<223> OTHER INFORMATION: Carboxyl-terminal half of heavy chain
      comprising the binding domain.

<400> SEQUENCE: 3

Met Pro Ile Thr Ile Asn Asn Phe Asn Tyr Ser Asp Pro Val Asp Asn
 1               5                  10                  15

Lys Asn Ile Leu Tyr Leu Asp Thr His Leu Asn Thr Leu Ala Asn Glu
            20                  25                  30

Pro Glu Lys Ala Phe Arg Ile Thr Gly Asn Ile Trp Val Ile Pro Asp
        35                  40                  45

Arg Phe Ser Arg Asn Ser Asn Pro Asn Leu Asn Lys Pro Pro Arg Val
    50                  55                  60

Thr Ser Pro Lys Ser Gly Tyr Tyr Asp Pro Asn Tyr Leu Ser Thr Asp
65                  70                  75                  80

Ser Asp Lys Asp Pro Phe Leu Lys Glu Ile Ile Lys Leu Phe Lys Arg
                85                  90                  95

Ile Asn Ser Arg Glu Ile Gly Glu Glu Leu Ile Tyr Arg Leu Ser Thr
            100                 105                 110

Asp Ile Pro Phe Pro Gly Asn Asn Asn Thr Pro Ile Asn Thr Phe Asp
        115                 120                 125

Phe Asp Val Asp Phe Asn Ser Val Asp Val Lys Thr Arg Gln Gly Asn
    130                 135                 140

Asn Trp Val Lys Thr Gly Ser Ile Asn Pro Ser Val Ile Ile Thr Gly
145                 150                 155                 160
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Pro Arg Glu Asn Ile Ile Asp Pro Glu Thr Ser Thr Phe Lys Leu Thr
                165                 170                 175

Asn Asn Thr Phe Ala Ala Gln Glu Gly Phe Gly Ala Leu Ser Ile Ile
            180                 185                 190

Ser Ile Ser Pro Arg Phe Met Leu Thr Tyr Ser Asn Ala Thr Asn Asp
        195                 200                 205

Val Gly Glu Gly Arg Phe Ser Lys Ser Glu Phe Cys Met Asp Pro Ile
    210                 215                 220

Leu Ile Leu Met His Glu Leu Asn His Ala Met His Asn Leu Tyr Gly
225                 230                 235                 240

Ile Ala Ile Pro Asn Asp Gln Thr Ile Ser Ser Val Thr Ser Asn Ile
                245                 250                 255

Phe Tyr Ser Gln Tyr Asn Val Lys Leu Glu Tyr Ala Glu Ile Tyr Ala
            260                 265                 270

Phe Gly Gly Pro Thr Ile Asp Leu Ile Pro Lys Ser Ala Arg Lys Tyr
        275                 280                 285

Phe Glu Glu Lys Ala Leu Asp Tyr Tyr Arg Ser Ile Ala Lys Arg Leu
    290                 295                 300

Asn Ser Ile Thr Thr Ala Asn Pro Ser Ser Phe Asn Lys Tyr Ile Gly
305                 310                 315                 320

Glu Tyr Lys Gln Lys Leu Ile Arg Lys Tyr Arg Phe Val Val Glu Ser
                325                 330                 335

Ser Gly Glu Val Thr Val Asn Arg Asn Lys Phe Val Glu Leu Tyr Asn
            340                 345                 350

Glu Leu Thr Gln Ile Phe Thr Glu Phe Asn Tyr Ala Lys Ile Tyr Asn
        355                 360                 365

Val Gln Asn Arg Lys Ile Tyr Leu Ser Asn Val Tyr Thr Pro Val Thr
    370                 375                 380

Ala Asn Ile Leu Asp Asp Asn Val Tyr Asp Ile Gln Asn Gly Phe Asn
385                 390                 395                 400

Ile Pro Lys Ser Asn Leu Asn Val Leu Phe Met Gly Gln Asn Leu Ser
                405                 410                 415

Arg Asn Pro Ala Leu Arg Lys Val Asn Pro Glu Asn Met Leu Tyr Leu
            420                 425                 430

Phe Thr Lys Phe Cys His Lys Ala Ile Asp Gly Arg Ser Leu Tyr Asn
        435                 440                 445

Lys Thr Leu Asp Cys Arg Glu Leu Leu Val Lys Asn Thr Asp Leu Pro
    450                 455                 460

Phe Ile Gly Asp Ile Ser Asp Val Lys Thr Asp Ile Phe Leu Arg Lys
465                 470                 475                 480

Asp Ile Asn Glu Glu Thr Glu Val Ile Tyr Tyr Pro Asp Asn Val Ser
                485                 490                 495

Val Asp Gln Val Ile Leu Ser Lys Asn Thr Ser Glu His Gly Gln Leu
            500                 505                 510

Asp Leu Leu Tyr Pro Ser Ile Asp Ser Glu Ser Glu Ile Leu Pro Gly
        515                 520                 525

Glu Asn Gln Val Phe Tyr Asp Asn Arg Thr Gln Asn Val Asp Tyr Leu
    530                 535                 540

Asn Ser Tyr Tyr Tyr Leu Glu Ser Gln Lys Leu Ser Asp Asn Val Glu
545                 550                 555                 560

Asp Phe Thr Phe Thr Arg Ser Ile Glu Glu Ala Leu Asp Asn Ser Ala
                565                 570                 575

Lys Val Tyr Thr Tyr Phe Pro Thr Leu Ala Asn Lys Val Asn Ala Gly
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            580                 585                 590

Val Gln Gly Gly Leu Phe Leu Met Trp Ala Asn Asp Val Val Glu Asp
        595                 600                 605

Phe Thr Thr Asn Ile Leu Arg Lys Asp Thr Leu Asp Lys Ile Ser Asp
    610                 615                 620

Val Ser Ala Ile Ile Pro Tyr Ile Gly Pro Ala Leu Asn Ile Ser Asn
625                 630                 635                 640

Ser Val Arg Arg Gly Asn Phe Thr Glu Ala Phe Ala Val Thr Gly Val
                645                 650                 655

Thr Ile Leu Leu Glu Ala Phe Pro Glu Phe Thr Ile Pro Ala Leu Gly
            660                 665                 670

Ala Phe Val Ile Tyr Ser Lys Val Gln Glu Arg Asn Glu Ile Ile Lys
        675                 680                 685

Thr Ile Asp Asn Cys Leu Glu Gln Arg Ile Lys Arg Trp Lys Asp Ser
    690                 695                 700

Tyr Glu Trp Met Met Gly Thr Trp Leu Ser Arg Ile Ile Thr Gln Phe
705                 710                 715                 720

Asn Asn Ile Ser Tyr Gln Met Tyr Asp Ser Leu Asn Tyr Gln Ala Gly
                725                 730                 735

Ala Ile Lys Ala Lys Ile Asp Leu Glu Tyr Lys Lys Tyr Ser Gly Ser
            740                 745                 750

Asp Lys Glu Asn Ile Lys Ser Gln Val Glu Asn Leu Lys Asn Ser Leu
        755                 760                 765

Asp Val Lys Ile Ser Glu Ala Met Asn Asn Ile Asn Lys Phe Ile Arg
    770                 775                 780

Glu Cys Ser Val Thr Tyr Leu Phe Lys Asn Met Leu Pro Lys Val Ile
785                 790                 795                 800

Asp Glu Leu Asn Glu Phe Asp Arg Asn Thr Lys Ala Lys Leu Ile Asn
                805                 810                 815

Leu Ile Asp Ser His Asn Ile Ile Leu Val Gly Glu Val Asp Lys Leu
            820                 825                 830

Lys Ala Lys Val Asn Asn Ser Phe Gln Asn Thr Ile Pro Phe Asn Ile
        835                 840                 845

Phe Ser Tyr Thr Asn Asn Ser Leu Leu Lys Asp Ile Ile Asn Glu Tyr
    850                 855                 860

Phe Asn Asn Ile Asn Asp Ser Lys Ile Leu Ser Leu Gln Asn Arg Lys
865                 870                 875                 880

Asn Thr Leu Val Asp Thr Ser Gly Tyr Asn Ala Glu Val Ser Glu Glu
                885                 890                 895

Gly Asp Val Gln Leu Asn Pro Ile Phe Pro Phe Asp Phe Lys Leu Gly
            900                 905                 910

Ser Ser Gly Glu Asp Arg Gly Lys Val Ile Val Thr Gln Asn Glu Asn
        915                 920                 925

Ile Val Tyr Asn Ser Met Tyr Glu Ser Phe Ser Ile Ser Phe Trp Ile
    930                 935                 940

Arg Ile Asn Lys Trp Val Ser Asn Leu Pro Gly Tyr Thr Ile Ile Asp
945                 950                 955                 960

Ser Val Lys Asn Asn Ser Gly Trp Ser Ile Gly Ile Ile Ser Asn Phe
                965                 970                 975

Leu Val Phe Thr Leu Lys Gln Asn Glu Asp Ser Glu Gln Ser Ile Asn
            980                 985                 990

Phe Ser Tyr Asp Ile Ser Asn Asn Ala Pro Gly Tyr Asn Lys Trp Phe
        995                 1000                1005
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Phe Val Thr Val Thr Asn Asn Met Met Gly Asn Met Lys Ile Tyr Ile
1010 1015 1020

Asn Gly Lys Leu Ile Asp Thr Ile Lys Val Lys Glu Leu Thr Gly Ile
1025 1030 1035 1040

Asn Phe Ser Lys Thr Ile Thr Phe Glu Ile Asn Lys Ile Pro Asp Thr
1045 1050 1055

Gly Leu Ile Thr Ser Asp Ser Asp Asn Ile Asn Met Trp Ile Arg Asp
1060 1065 1070

Phe Tyr Ile Phe Ala Lys Glu Leu Asp Gly Lys Asp Ile Asn Ile Leu
1075 1080 1085

Phe Asn Ser Leu Gln Tyr Thr Asn Val Val Lys Asp Tyr Trp Gly Asn
1090 1095 1100

Asp Leu Arg Tyr Asn Lys Glu Tyr Tyr Met Val Asn Ile Asp Tyr Leu
1105 1110 1115 1120

Asn Arg Tyr Met Tyr Ala Asn Ser Arg Gln Ile Val Phe Asn Thr Arg
1125 1130 1135

Arg Asn Asn Asn Asp Phe Asn Glu Gly Tyr Lys Ile Ile Ile Lys Arg
1140 1145 1150

Ile Arg Gly Asn Thr Asn Asp Thr Arg Val Arg Gly Gly Asp Ile Leu
1155 1160 1165

Tyr Phe Asp Met Thr Ile Asn Asn Lys Ala Tyr Asn Leu Phe Met Lys
1170 1175 1180

Asn Glu Thr Met Tyr Ala Asp Asn His Ser Thr Glu Asp Ile Tyr Ala
1185 1190 1195 1200

Ile Gly Leu Arg Glu Gln Thr Lys Asp Ile Asn Asp Asn Ile Ile Phe
1205 1210 1215

Gln Ile Gln Pro Met Asn Asn Thr Tyr Tyr Tyr Ala Ser Gln Ile Phe
1220 1225 1230

Lys Ser Asn Phe Asn Gly Glu Asn Ile Ser Gly Ile Cys Ser Ile Gly
1235 1240 1245

Thr Tyr Arg Phe Arg Leu Gly Gly Asp Trp Tyr Arg His Asn Tyr Leu
1250 1255 1260

Val Pro Thr Val Lys Gln Gly Asn Tyr Ala Ser Leu Leu Glu Ser Thr
1265 1270 1275 1280

Ser Thr His Trp Gly Phe Val Pro Val Ser Glu
1285 1290

<210> SEQ ID NO 4
<211> LENGTH: 1276
<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum Serotype D
<220> FEATURE:
<221> NAME/KEY: DOMAIN
<222> LOCATION: (1)...(442)
<223> OTHER INFORMATION: Light chain comprising the enzymatic domain.
<221> NAME/KEY: DOMAIN
<222> LOCATION: (443)...(851)
<223> OTHER INFORMATION: Amino-terminal half of heavy chain comprising
the translocation domain.
<221> NAME/KEY: DOMAIN
<222> LOCATION: (852)...(1276)
<223> OTHER INFORMATION: Carboxyl-terminal half of heavy chain
comprising the binding domain.

<400> SEQUENCE: 4

Met Thr Trp Pro Val Lys Asp Phe Asn Tyr Ser Asp Pro Val Asn Asp
1 5 10 15

Asn Asp Ile Leu Tyr Leu Arg Ile Pro Gln Asn Lys Leu Ile Thr Thr

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            20                  25                  30

Pro Val Lys Ala Phe Met Ile Thr Gln Asn Ile Trp Val Ile Pro Glu
        35                  40                  45

Arg Phe Ser Ser Asp Thr Asn Pro Ser Leu Ser Lys Pro Pro Arg Pro
    50                  55                  60

Thr Ser Lys Tyr Gln Ser Tyr Tyr Asp Pro Ser Tyr Leu Ser Thr Asp
65                  70                  75                  80

Glu Gln Lys Asp Thr Phe Leu Lys Gly Ile Ile Lys Leu Phe Lys Arg
                85                  90                  95

Ile Asn Glu Arg Asp Ile Gly Lys Lys Leu Ile Asn Tyr Leu Val Val
            100                 105                 110

Gly Ser Pro Phe Met Gly Asp Ser Ser Thr Pro Glu Asp Thr Phe Asp
        115                 120                 125

Phe Thr Arg His Thr Thr Asn Ile Ala Val Glu Lys Phe Glu Asn Gly
    130                 135                 140

Ser Trp Lys Val Thr Asn Ile Ile Thr Pro Ser Val Leu Ile Phe Gly
145                 150                 155                 160

Pro Leu Pro Asn Ile Leu Asp Tyr Thr Ala Ser Leu Thr Leu Gln Gly
                165                 170                 175

Gln Gln Ser Asn Pro Ser Phe Glu Gly Phe Gly Thr Leu Ser Ile Leu
            180                 185                 190

Lys Val Ala Pro Glu Phe Leu Leu Thr Phe Ser Asp Val Thr Ser Asn
        195                 200                 205

Gln Ser Ser Ala Val Leu Gly Lys Ser Ile Phe Cys Met Asp Pro Val
    210                 215                 220

Ile Ala Leu Met His Glu Leu Thr His Ser Leu His Gln Leu Tyr Gly
225                 230                 235                 240

Ile Asn Ile Pro Ser Asp Lys Arg Ile Arg Pro Gln Val Ser Glu Gly
                245                 250                 255

Phe Phe Ser Gln Asp Gly Pro Asn Val Gln Phe Glu Glu Leu Tyr Thr
            260                 265                 270

Phe Gly Gly Leu Asp Val Glu Ile Ile Pro Gln Ile Glu Arg Ser Gln
        275                 280                 285

Leu Arg Glu Lys Ala Leu Gly His Tyr Lys Asp Ile Ala Lys Arg Leu
    290                 295                 300

Asn Asn Ile Asn Lys Thr Ile Pro Ser Ser Trp Ile Ser Asn Ile Asp
305                 310                 315                 320

Lys Tyr Lys Lys Ile Phe Ser Glu Lys Tyr Asn Phe Asp Lys Asp Asn
                325                 330                 335

Thr Gly Asn Phe Val Val Asn Ile Asp Lys Phe Asn Ser Leu Tyr Ser
            340                 345                 350

Asp Leu Thr Asn Val Met Ser Glu Val Val Tyr Ser Ser Gln Tyr Asn
        355                 360                 365

Val Lys Asn Arg Thr His Tyr Phe Ser Arg His Tyr Leu Pro Val Phe
    370                 375                 380

Ala Asn Ile Leu Asp Asp Asn Ile Tyr Thr Ile Arg Asp Gly Phe Asn
385                 390                 395                 400

Leu Thr Asn Lys Gly Phe Asn Ile Glu Asn Ser Gly Gln Asn Ile Glu
                405                 410                 415

Arg Asn Pro Ala Leu Gln Lys Leu Ser Ser Glu Ser Val Val Asp Leu
            420                 425                 430

Phe Thr Lys Val Cys Leu Arg Leu Thr Lys Asn Ser Arg Asp Asp Ser
        435                 440                 445
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Thr Cys Ile Lys Val Lys Asn Asn Arg Leu Pro Tyr Val Ala Asp Lys
    450                 455                 460

Asp Ser Ile Ser Gln Glu Ile Phe Glu Asn Lys Ile Ile Thr Asp Glu
465                 470                 475                 480

Thr Asn Val Gln Asn Tyr Ser Asp Lys Phe Ser Leu Asp Glu Ser Ile
                485                 490                 495

Leu Asp Gly Gln Val Pro Ile Asn Pro Glu Ile Val Asp Pro Leu Leu
            500                 505                 510

Pro Asn Val Asn Met Glu Pro Leu Asn Leu Pro Gly Glu Glu Ile Val
        515                 520                 525

Phe Tyr Asp Asp Ile Thr Lys Tyr Val Asp Tyr Leu Asn Ser Tyr Tyr
    530                 535                 540

Tyr Leu Glu Ser Gln Lys Leu Ser Asn Asn Val Glu Asn Ile Thr Leu
545                 550                 555                 560

Thr Thr Ser Val Glu Glu Ala Leu Gly Tyr Ser Asn Lys Ile Tyr Thr
                565                 570                 575

Phe Leu Pro Ser Leu Ala Glu Lys Val Asn Lys Gly Val Gln Ala Gly
            580                 585                 590

Leu Phe Leu Asn Trp Ala Asn Glu Val Val Glu Asp Phe Thr Thr Asn
        595                 600                 605

Ile Met Lys Lys Asp Thr Leu Asp Lys Ile Ser Asp Val Ser Val Ile
    610                 615                 620

Ile Pro Tyr Ile Gly Pro Ala Leu Asn Ile Gly Asn Ser Ala Leu Arg
625                 630                 635                 640

Gly Asn Phe Asn Gln Ala Phe Ala Thr Ala Gly Val Ala Phe Leu Leu
                645                 650                 655

Glu Gly Phe Pro Glu Phe Thr Ile Pro Ala Leu Gly Val Phe Thr Phe
            660                 665                 670

Tyr Ser Ser Ile Gln Glu Arg Glu Lys Ile Ile Lys Thr Ile Glu Asn
        675                 680                 685

Cys Leu Glu Gln Arg Val Lys Arg Trp Lys Asp Ser Tyr Gln Trp Met
    690                 695                 700

Val Ser Asn Trp Leu Ser Arg Ile Thr Thr Gln Phe Asn His Ile Asn
705                 710                 715                 720

Tyr Gln Met Tyr Asp Ser Leu Ser Tyr Gln Ala Asp Ala Ile Lys Ala
                725                 730                 735

Lys Ile Asp Leu Glu Tyr Lys Lys Tyr Ser Gly Ser Asp Lys Glu Asn
            740                 745                 750

Ile Lys Ser Gln Val Glu Asn Leu Lys Asn Ser Leu Asp Val Lys Ile
        755                 760                 765

Ser Glu Ala Met Asn Asn Ile Asn Lys Phe Ile Arg Glu Cys Ser Val
    770                 775                 780

Thr Tyr Leu Phe Lys Asn Met Leu Pro Lys Val Ile Asp Glu Leu Asn
785                 790                 795                 800

Lys Phe Asp Leu Arg Thr Lys Thr Glu Leu Ile Asn Leu Ile Asp Ser
                805                 810                 815

His Asn Ile Ile Leu Val Gly Glu Val Asp Arg Leu Lys Ala Lys Val
            820                 825                 830

Asn Glu Ser Phe Glu Asn Thr Met Pro Phe Asn Ile Phe Ser Tyr Thr
        835                 840                 845

Asn Asn Ser Leu Leu Lys Asp Ile Ile Asn Glu Tyr Phe Asn Ser Ile
    850                 855                 860
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Asn Asp Ser Lys Ile Leu Ser Leu Gln Asn Lys Lys Asn Ala Leu Val
865                 870                 875                 880

Asp Thr Ser Gly Tyr Asn Ala Glu Val Arg Val Gly Asp Asn Val Gln
                885                 890                 895

Leu Asn Thr Ile Tyr Thr Asn Asp Phe Lys Leu Ser Ser Ser Gly Asp
            900                 905                 910

Lys Ile Ile Val Asn Leu Asn Asn Asn Ile Leu Tyr Ser Ala Ile Tyr
        915                 920                 925

Glu Asn Ser Ser Val Ser Phe Trp Ile Lys Ile Ser Lys Asp Leu Thr
    930                 935                 940

Asn Ser His Asn Glu Tyr Thr Ile Ile Asn Ser Ile Glu Gln Asn Ser
945                 950                 955                 960

Gly Trp Lys Leu Cys Ile Arg Asn Gly Asn Ile Glu Trp Ile Leu Gln
                965                 970                 975

Asp Val Asn Arg Lys Tyr Lys Ser Leu Ile Phe Asp Tyr Ser Glu Ser
            980                 985                 990

Leu Ser His Thr Gly Tyr Thr Asn Lys Trp Phe Phe Val Thr Ile Thr
        995                 1000                1005

Asn Asn Ile Met Gly Tyr Met Lys Leu Tyr Ile Asn Gly Glu Leu Lys
    1010                1015                1020

Gln Ser Gln Lys Ile Glu Asp Leu Asp Glu Val Lys Leu Asp Lys Thr
1025                1030                1035                1040

Ile Val Phe Gly Ile Asp Glu Asn Ile Asp Glu Asn Gln Met Leu Trp
                1045                1050                1055

Ile Arg Asp Phe Asn Ile Phe Ser Lys Glu Leu Ser Asn Glu Asp Ile
            1060                1065                1070

Asn Ile Val Tyr Glu Gly Gln Ile Leu Arg Asn Val Ile Lys Asp Tyr
        1075                1080                1085

Trp Gly Asn Pro Leu Lys Phe Asp Thr Glu Tyr Tyr Ile Ile Asn Asp
    1090                1095                1100

Asn Tyr Ile Asp Arg Tyr Ile Ala Pro Glu Ser Asn Val Leu Val Leu
1105                1110                1115                1120

Val Gln Tyr Pro Asp Arg Ser Lys Leu Tyr Thr Gly Asn Pro Ile Thr
                1125                1130                1135

Ile Lys Ser Val Ser Asp Lys Asn Pro Tyr Ser Arg Ile Leu Asn Gly
            1140                1145                1150

Asp Asn Ile Ile Leu His Met Leu Tyr Asn Ser Arg Lys Tyr Met Ile
        1155                1160                1165

Ile Arg Asp Thr Asp Thr Ile Tyr Ala Thr Gln Gly Gly Glu Cys Ser
    1170                1175                1180

Gln Asn Cys Val Tyr Ala Leu Lys Leu Gln Ser Asn Leu Gly Asn Tyr
1185                1190                1195                1200

Gly Ile Gly Ile Phe Ser Ile Lys Asn Ile Val Ser Lys Asn Lys Tyr
                1205                1210                1215

Cys Ser Gln Ile Phe Ser Ser Phe Arg Glu Asn Thr Met Leu Leu Ala
            1220                1225                1230

Asp Ile Tyr Lys Pro Trp Arg Phe Ser Phe Lys Asn Ala Tyr Thr Pro
        1235                1240                1245

Val Ala Val Thr Asn Tyr Glu Thr Lys Leu Leu Ser Thr Ser Ser Phe
    1250                1255                1260

Trp Lys Phe Ile Ser Arg Asp Pro Gly Trp Val Glu
1265                1270                1275
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<210> SEQ ID NO 5
<211> LENGTH: 1252
<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum Serotype E
<220> FEATURE:
<221> NAME/KEY: DOMAIN
<222> LOCATION: (1)...(422)
<223> OTHER INFORMATION: Light chain comprising the enzymatic domain.
<221> NAME/KEY: DOMAIN
<222> LOCATION: (423)...(834)
<223> OTHER INFORMATION: Amino-terminal half of heavy chain comprising
the translocation domain.
<221> NAME/KEY: DOMAIN
<222> LOCATION: (835)...(1252)
<223> OTHER INFORMATION: Carboxyl-terminal half of heavy chain
comprising the binding domain.

<400> SEQUENCE: 5

Met Pro Lys Ile Asn Ser Phe Asn Tyr Asn Asp Pro Val Asn Asp Arg
1 5 10 15

Thr Ile Leu Tyr Ile Lys Pro Gly Gly Cys Gln Glu Phe Tyr Lys Ser
20 25 30

Phe Asn Ile Met Lys Asn Ile Trp Ile Ile Pro Glu Arg Asn Val Ile
35 40 45

Gly Thr Thr Pro Gln Asp Phe His Pro Pro Thr Ser Leu Lys Asn Gly
50 55 60

Asp Ser Ser Tyr Tyr Asp Pro Asn Tyr Leu Gln Ser Asp Glu Glu Lys
65 70 75 80

Asp Arg Phe Leu Lys Ile Val Thr Lys Ile Phe Asn Arg Ile Asn Asn
85 90 95

Asn Leu Ser Gly Gly Ile Leu Leu Glu Glu Leu Ser Lys Ala Asn Pro
100 105 110

Tyr Leu Gly Asn Asp Asn Thr Pro Asp Asn Gln Phe His Ile Gly Asp
115 120 125

Ala Ser Ala Val Glu Ile Lys Phe Ser Asn Gly Ser Gln Asp Ile Leu
130 135 140

Leu Pro Asn Val Ile Ile Met Gly Ala Glu Pro Asp Leu Phe Glu Thr
145 150 155 160

Asn Ser Ser Asn Ile Ser Leu Arg Asn Asn Tyr Met Pro Ser Asn His
165 170 175

Gly Phe Gly Ser Ile Ala Ile Val Thr Phe Ser Pro Glu Tyr Ser Phe
180 185 190

Arg Phe Asn Asp Asn Ser Met Asn Glu Phe Ile Gln Asp Pro Ala Leu
195 200 205

Thr Leu Met His Glu Leu Ile His Ser Leu His Gly Leu Tyr Gly Ala
210 215 220

Lys Gly Ile Thr Thr Lys Tyr Thr Ile Thr Gln Lys Gln Asn Pro Leu
225 230 235 240

Ile Thr Asn Ile Arg Gly Thr Asn Ile Glu Glu Phe Leu Thr Phe Gly
245 250 255

Gly Thr Asp Leu Asn Ile Ile Thr Ser Ala Gln Ser Asn Asp Ile Tyr
260 265 270

Thr Asn Leu Leu Ala Asp Tyr Lys Lys Ile Ala Ser Lys Leu Ser Lys
275 280 285

Val Gln Val Ser Asn Pro Leu Leu Asn Pro Tyr Lys Asp Val Phe Glu
290 295 300

Ala Lys Tyr Gly Leu Asp Lys Asp Ala Ser Gly Ile Tyr Ser Val Asn
305 310 315 320

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Ile Asn Lys Phe Asn Asp Ile Phe Lys Lys Leu Tyr Ser Phe Thr Glu
                325                 330                 335

Phe Asp Leu Ala Thr Lys Phe Gln Val Lys Cys Arg Gln Thr Tyr Ile
            340                 345                 350

Gly Gln Tyr Lys Tyr Phe Lys Leu Ser Asn Leu Leu Asn Asp Ser Ile
        355                 360                 365

Tyr Asn Ile Ser Glu Gly Tyr Asn Ile Asn Asn Leu Lys Val Asn Phe
    370                 375                 380

Arg Gly Gln Asn Ala Asn Leu Asn Pro Arg Ile Ile Thr Pro Ile Thr
385                 390                 395                 400

Gly Arg Gly Leu Val Lys Lys Ile Ile Arg Phe Cys Lys Asn Ile Val
                405                 410                 415

Ser Val Lys Gly Ile Arg Lys Ser Ile Cys Ile Glu Ile Asn Asn Gly
            420                 425                 430

Glu Leu Phe Phe Val Ala Ser Glu Asn Ser Tyr Asn Asp Asp Asn Ile
        435                 440                 445

Asn Thr Pro Lys Glu Ile Asp Asp Thr Val Thr Ser Asn Asn Asn Tyr
    450                 455                 460

Glu Asn Asp Leu Asp Gln Val Ile Leu Asn Phe Asn Ser Glu Ser Ala
465                 470                 475                 480

Pro Gly Leu Ser Asp Glu Lys Leu Asn Leu Thr Ile Gln Asn Asp Ala
                485                 490                 495

Tyr Ile Pro Lys Tyr Asp Ser Asn Gly Thr Ser Asp Ile Glu Gln His
            500                 505                 510

Asp Val Asn Glu Leu Asn Val Phe Phe Tyr Leu Asp Ala Gln Lys Val
        515                 520                 525

Pro Glu Gly Glu Asn Asn Val Asn Leu Thr Ser Ser Ile Asp Thr Ala
    530                 535                 540

Leu Leu Glu Gln Pro Lys Ile Tyr Thr Phe Phe Ser Ser Glu Phe Ile
545                 550                 555                 560

Asn Asn Val Asn Lys Pro Val Gln Ala Ala Leu Phe Val Ser Trp Ile
                565                 570                 575

Gln Gln Val Leu Val Asp Phe Thr Thr Glu Ala Asn Gln Lys Ser Thr
            580                 585                 590

Val Asp Lys Ile Ala Asp Ile Ser Ile Val Val Pro Tyr Ile Gly Leu
        595                 600                 605

Ala Leu Asn Ile Gly Asn Glu Ala Gln Lys Gly Asn Phe Lys Asp Ala
    610                 615                 620

Leu Glu Leu Leu Gly Ala Gly Ile Leu Leu Glu Phe Glu Pro Glu Leu
625                 630                 635                 640

Leu Ile Pro Thr Ile Leu Val Phe Thr Ile Lys Ser Phe Leu Gly Ser
                645                 650                 655

Ser Asp Asn Lys Asn Lys Val Ile Lys Ala Ile Asn Asn Ala Leu Lys
            660                 665                 670

Glu Arg Asp Glu Lys Trp Lys Glu Val Tyr Ser Phe Ile Val Ser Asn
        675                 680                 685

Trp Met Thr Lys Ile Asn Thr Gln Phe Asn Lys Arg Lys Glu Gln Met
    690                 695                 700

Tyr Gln Ala Leu Gln Asn Gln Val Asn Ala Ile Lys Thr Ile Ile Glu
705                 710                 715                 720

Ser Lys Tyr Asn Ser Tyr Thr Leu Glu Glu Lys Asn Glu Leu Thr Asn
                725                 730                 735

Lys Tyr Asp Ile Lys Gln Ile Glu Asn Glu Leu Asn Gln Lys Val Ser
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            740                 745                 750

Ile Ala Met Asn Asn Ile Asp Arg Phe Leu Thr Glu Ser Ser Ile Ser
        755                 760                 765

Tyr Leu Met Lys Leu Ile Asn Glu Val Lys Ile Asn Lys Leu Arg Glu
    770                 775                 780

Tyr Asp Glu Asn Val Lys Thr Tyr Leu Leu Asn Tyr Ile Ile Gln His
785                 790                 795                 800

Gly Ser Ile Leu Gly Glu Ser Gln Gln Glu Leu Asn Ser Met Val Thr
                805                 810                 815

Asp Thr Leu Asn Asn Ser Ile Pro Phe Lys Leu Ser Ser Tyr Thr Asp
            820                 825                 830

Asp Lys Ile Leu Ile Ser Tyr Phe Asn Lys Phe Phe Lys Arg Ile Lys
        835                 840                 845

Ser Ser Ser Val Leu Asn Met Arg Tyr Lys Asn Asp Lys Tyr Val Asp
    850                 855                 860

Thr Ser Gly Tyr Asp Ser Asn Ile Asn Ile Asn Gly Asp Val Tyr Lys
865                 870                 875                 880

Tyr Pro Thr Asn Lys Asn Gln Phe Gly Ile Tyr Asn Asp Lys Leu Ser
                885                 890                 895

Glu Val Asn Ile Ser Gln Asn Asp Tyr Ile Ile Tyr Asp Asn Lys Tyr
            900                 905                 910

Lys Asn Phe Ser Ile Ser Phe Trp Val Arg Ile Pro Asn Tyr Asp Asn
        915                 920                 925

Lys Ile Val Asn Val Asn Asn Glu Tyr Thr Ile Ile Asn Cys Met Arg
    930                 935                 940

Asp Asn Asn Ser Gly Trp Lys Val Ser Leu Asn His Asn Glu Ile Ile
945                 950                 955                 960

Trp Thr Leu Gln Asp Asn Ala Gly Ile Asn Gln Lys Leu Ala Phe Asn
                965                 970                 975

Tyr Gly Asn Ala Asn Gly Ile Ser Asp Tyr Ile Asn Lys Trp Ile Phe
            980                 985                 990

Val Thr Ile Thr Asn Asp Arg Leu Gly Asp Ser Lys Leu Tyr Ile Asn
        995                 1000                1005

Gly Asn Leu Ile Asp Gln Lys Ser Ile Leu Asn Leu Gly Asn Ile His
    1010                1015                1020

Val Ser Asp Asn Ile Leu Phe Lys Ile Val Asn Cys Ser Tyr Thr Arg
1025                1030                1035                1040

Tyr Ile Gly Ile Arg Tyr Phe Asn Ile Phe Asp Lys Glu Leu Asp Glu
                1045                1050                1055

Thr Glu Ile Gln Thr Leu Tyr Ser Asn Glu Pro Asn Thr Asn Ile Leu
            1060                1065                1070

Lys Asp Phe Trp Gly Asn Tyr Leu Leu Tyr Asp Lys Glu Tyr Tyr Leu
        1075                1080                1085

Leu Asn Val Leu Lys Pro Asn Asn Phe Ile Asp Arg Arg Lys Asp Ser
    1090                1095                1100

Thr Leu Ser Ile Asn Asn Ile Arg Ser Thr Ile Leu Leu Ala Asn Arg
1105                1110                1115                1120

Leu Tyr Ser Gly Ile Lys Val Lys Ile Gln Arg Val Asn Asn Ser Ser
                1125                1130                1135

Thr Asn Asp Asn Leu Val Arg Lys Asn Asp Gln Val Tyr Ile Asn Phe
            1140                1145                1150

Val Ala Ser Lys Thr His Leu Phe Pro Leu Tyr Ala Asp Thr Ala Thr
        1155                1160                1165
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Thr Asn Lys Glu Lys Thr Ile Lys Ile Ser Ser Ser Gly Asn Arg Phe
    1170                1175                1180

Asn Gln Val Val Val Met Asn Ser Val Gly Asn Asn Cys Thr Met Asn
1185                1190                1195                1200

Phe Lys Asn Asn Asn Gly Asn Asn Ile Gly Leu Leu Gly Phe Lys Ala
                1205                1210                1215

Asp Thr Val Val Ala Ser Thr Trp Tyr Tyr Thr His Met Arg Asp His
            1220                1225                1230

Thr Asn Ser Asn Gly Cys Phe Trp Asn Phe Ile Ser Glu Glu His Gly
        1235                1240                1245

Trp Gln Glu Lys
    1250
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<210> SEQ ID NO 6
<211> LENGTH: 1274
<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum Serotype F
<220> FEATURE:
<221> NAME/KEY: DOMAIN
<222> LOCATION: (1)...(436)
<223> OTHER INFORMATION: Light chain comprising the enzymatic domain.
<221> NAME/KEY: DOMAIN
<222> LOCATION: (437)...(852)
<223> OTHER INFORMATION: Amino-terminal half of heavy chain comprising
      the translocation domain.
<221> NAME/KEY: DOMAIN
<222> LOCATION: (853)...(1274)
<223> OTHER INFORMATION: Carboxyl-terminal half of heavy chain
      comprising the binding domain.

<400> SEQUENCE: 6

Met Pro Val Ala Ile Asn Ser Phe Asn Tyr Asn Asp Pro Val Asn Asp
 1               5                  10                  15

Asp Thr Ile Leu Tyr Met Gln Ile Pro Tyr Glu Glu Lys Ser Lys Lys
            20                  25                  30

Tyr Tyr Lys Ala Phe Glu Ile Met Arg Asn Val Trp Ile Ile Pro Glu
        35                  40                  45

Arg Asn Thr Ile Gly Thr Asn Pro Ser Asp Phe Asp Pro Pro Ala Ser
    50                  55                  60

Leu Lys Asn Gly Ser Ser Ala Tyr Tyr Asp Pro Asn Tyr Leu Thr Thr
65                  70                  75                  80

Asp Ala Glu Lys Asp Arg Tyr Leu Lys Thr Thr Ile Lys Leu Phe Lys
                85                  90                  95

Arg Ile Asn Ser Asn Pro Ala Gly Lys Val Leu Leu Gln Glu Ile Ser
            100                 105                 110

Tyr Ala Lys Pro Tyr Leu Gly Asn Asp His Thr Pro Ile Asp Glu Phe
        115                 120                 125

Ser Pro Val Thr Arg Thr Thr Ser Val Asn Ile Lys Leu Ser Thr Asn
    130                 135                 140

Val Glu Ser Ser Met Leu Leu Asn Leu Leu Val Leu Gly Ala Gly Pro
145                 150                 155                 160

Asp Ile Phe Glu Ser Cys Cys Tyr Pro Val Arg Lys Leu Ile Asp Pro
                165                 170                 175

Asp Val Val Tyr Asp Pro Ser Asn Tyr Gly Phe Gly Ser Ile Asn Ile
            180                 185                 190

Val Thr Phe Ser Pro Glu Tyr Glu Tyr Thr Phe Asn Asp Ile Ser Gly
        195                 200                 205

Gly His Asn Ser Ser Thr Glu Ser Phe Ile Ala Asp Pro Ala Ile Ser
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    210                 215                 220

Leu Ala His Glu Leu Ile His Ala Leu His Gly Leu Tyr Gly Ala Arg
225                 230                 235                 240

Gly Val Thr Tyr Glu Glu Thr Ile Glu Val Lys Gln Ala Pro Leu Met
                245                 250                 255

Ile Ala Glu Lys Pro Ile Arg Leu Glu Glu Phe Leu Thr Phe Gly Gly
            260                 265                 270

Gln Asp Leu Asn Ile Ile Thr Ser Ala Met Lys Glu Lys Ile Tyr Asn
        275                 280                 285

Asn Leu Leu Ala Asn Tyr Glu Lys Ile Ala Thr Arg Leu Ser Glu Val
    290                 295                 300

Asn Ser Ala Pro Pro Glu Tyr Asp Ile Asn Glu Tyr Lys Asp Tyr Phe
305                 310                 315                 320

Gln Trp Lys Tyr Gly Leu Asp Lys Asn Ala Asp Gly Ser Tyr Thr Val
                325                 330                 335

Asn Glu Asn Lys Phe Asn Glu Ile Tyr Lys Lys Leu Tyr Ser Phe Thr
            340                 345                 350

Glu Ser Asp Leu Ala Asn Lys Phe Lys Val Lys Cys Arg Asn Thr Tyr
        355                 360                 365

Phe Ile Lys Tyr Glu Phe Leu Lys Val Pro Asn Leu Leu Asp Asp Asp
    370                 375                 380

Ile Tyr Thr Val Ser Glu Gly Phe Asn Ile Gly Asn Leu Ala Val Asn
385                 390                 395                 400

Asn Arg Gly Gln Ser Ile Lys Leu Asn Pro Lys Ile Ile Asp Ser Ile
                405                 410                 415

Pro Asp Lys Gly Leu Val Glu Lys Ile Val Lys Phe Cys Lys Ser Val
            420                 425                 430

Ile Pro Arg Lys Gly Thr Lys Ala Pro Pro Arg Leu Cys Ile Arg Val
        435                 440                 445

Asn Asn Ser Glu Leu Phe Phe Val Ala Ser Glu Ser Ser Tyr Asn Glu
    450                 455                 460

Asn Asp Ile Asn Thr Pro Lys Glu Ile Asp Asp Thr Thr Asn Leu Asn
465                 470                 475                 480

Asn Asn Tyr Arg Asn Asn Leu Asp Glu Val Ile Leu Asp Tyr Asn Ser
                485                 490                 495

Gln Thr Ile Pro Gln Ile Ser Asn Arg Thr Leu Asn Thr Leu Val Gln
            500                 505                 510

Asp Asn Ser Tyr Val Pro Arg Tyr Asp Ser Asn Gly Thr Ser Glu Ile
        515                 520                 525

Glu Glu Tyr Asp Val Val Asp Phe Asn Val Phe Phe Tyr Leu His Ala
    530                 535                 540

Gln Lys Val Pro Glu Gly Glu Thr Asn Ile Ser Leu Thr Ser Ser Ile
545                 550                 555                 560

Asp Thr Ala Leu Leu Glu Glu Ser Lys Asp Ile Phe Phe Ser Ser Glu
                565                 570                 575

Phe Ile Asp Thr Ile Asn Lys Pro Val Asn Ala Ala Leu Phe Ile Asp
            580                 585                 590

Trp Ile Ser Lys Val Ile Arg Asp Phe Thr Thr Glu Ala Thr Gln Lys
        595                 600                 605

Ser Thr Val Asp Lys Ile Ala Asp Ile Ser Leu Ile Val Pro Tyr Val
    610                 615                 620

Gly Leu Ala Leu Asn Ile Ile Ile Glu Ala Glu Lys Gly Asn Phe Glu
625                 630                 635                 640
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Glu Ala Phe Glu Leu Leu Gly Val Gly Ile Leu Leu Glu Phe Val Pro
                645                 650                 655

Glu Leu Thr Ile Pro Val Ile Leu Val Phe Thr Ile Lys Ser Tyr Ile
            660                 665                 670

Asp Ser Tyr Glu Asn Lys Asn Lys Ala Ile Lys Ala Ile Asn Asn Ser
        675                 680                 685

Leu Ile Glu Arg Glu Ala Lys Trp Lys Glu Ile Tyr Ser Trp Ile Val
    690                 695                 700

Ser Asn Trp Leu Thr Arg Ile Asn Thr Gln Phe Asn Lys Arg Lys Glu
705                 710                 715                 720

Gln Met Tyr Gln Ala Leu Gln Asn Gln Val Asp Ala Ile Lys Thr Ala
                725                 730                 735

Ile Glu Tyr Lys Tyr Asn Asn Tyr Thr Ser Asp Glu Lys Asn Arg Leu
            740                 745                 750

Glu Ser Glu Tyr Asn Ile Asn Asn Ile Glu Glu Glu Leu Asn Lys Lys
        755                 760                 765

Val Ser Leu Ala Met Lys Asn Ile Glu Arg Phe Met Thr Glu Ser Ser
    770                 775                 780

Ile Ser Tyr Leu Met Lys Leu Ile Asn Glu Ala Lys Val Gly Lys Leu
785                 790                 795                 800

Lys Lys Tyr Asp Asn His Val Lys Ser Asp Leu Leu Asn Tyr Ile Leu
                805                 810                 815

Asp His Arg Ser Ile Leu Gly Glu Gln Thr Asn Glu Leu Ser Asp Leu
            820                 825                 830

Val Thr Ser Thr Leu Asn Ser Ser Ile Pro Phe Glu Leu Ser Ser Tyr
        835                 840                 845

Thr Asn Asp Lys Ile Leu Ile Ile Tyr Phe Asn Arg Leu Tyr Lys Lys
    850                 855                 860

Ile Lys Asp Ser Ser Ile Leu Asp Met Arg Tyr Glu Asn Asn Lys Phe
865                 870                 875                 880

Ile Asp Ile Ser Gly Tyr Gly Ser Asn Ile Ser Ile Asn Gly Asn Val
                885                 890                 895

Tyr Ile Tyr Ser Thr Asn Arg Asn Gln Phe Gly Ile Tyr Asn Ser Arg
            900                 905                 910

Leu Ser Glu Val Asn Ile Ala Gln Asn Asn Asp Ile Ile Tyr Asn Ser
        915                 920                 925

Arg Tyr Gln Asn Phe Ser Ile Ser Phe Trp Val Arg Ile Pro Lys His
    930                 935                 940

Tyr Lys Pro Met Asn His Asn Arg Glu Tyr Thr Ile Ile Asn Cys Met
945                 950                 955                 960

Gly Asn Asn Asn Ser Gly Trp Lys Ile Ser Leu Arg Thr Val Arg Asp
                965                 970                 975

Cys Glu Ile Ile Trp Thr Leu Gln Asp Thr Ser Gly Asn Lys Glu Asn
            980                 985                 990

Leu Ile Phe Arg Tyr Glu Glu Leu Asn Arg Ile Ser Asn Tyr Ile Asn
        995                 1000                1005

Lys Trp Ile Phe Val Thr Ile Thr Asn Asn Arg Leu Gly Asn Ser Arg
    1010                1015                1020

Ile Tyr Ile Asn Gly Asn Leu Ile Val Glu Lys Ser Ile Ser Asn Leu
1025                1030                1035                1040

Gly Asp Ile His Val Ser Asp Asn Ile Leu Phe Lys Ile Val Gly Cys
                1045                1050                1055
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Asp Asp Glu Thr Tyr Val Gly Ile Arg Tyr Phe Lys Val Phe Asn Thr
            1060                1065                1070

Glu Leu Asp Lys Thr Glu Ile Glu Thr Leu Tyr Ser Asn Glu Pro Asp
        1075                1080                1085

Pro Ser Ile Leu Lys Asn Tyr Trp Gly Asn Tyr Leu Leu Tyr Asn Lys
    1090                1095                1100

Lys Tyr Tyr Leu Phe Asn Leu Leu Arg Lys Asp Lys Tyr Ile Thr Leu
1105                1110                1115                1120

Asn Ser Gly Ile Leu Asn Ile Asn Gln Gln Arg Gly Val Thr Glu Gly
                1125                1130                1135

Ser Val Phe Leu Asn Tyr Lys Leu Tyr Glu Gly Val Glu Val Ile Ile
            1140                1145                1150

Arg Lys Asn Gly Pro Ile Asp Ile Ser Asn Thr Asp Asn Phe Val Arg
        1155                1160                1165

Lys Asn Asp Leu Ala Tyr Ile Asn Val Val Asp Arg Gly Val Glu Tyr
    1170                1175                1180

Arg Leu Tyr Ala Asp Thr Lys Ser Glu Lys Glu Lys Ile Ile Arg Thr
1185                1190                1195                1200

Ser Asn Leu Asn Asp Ser Leu Gly Gln Ile Ile Val Met Asp Ser Ile
                1205                1210                1215

Gly Asn Asn Cys Thr Met Asn Phe Gln Asn Asn Asn Gly Ser Asn Ile
            1220                1225                1230

Gly Leu Leu Gly Phe His Ser Asn Asn Leu Val Ala Ser Ser Trp Tyr
        1235                1240                1245

Tyr Asn Asn Ile Arg Arg Asn Thr Ser Ser Asn Gly Cys Phe Trp Ser
    1250                1255                1260

Ser Ile Ser Lys Glu Asn Gly Trp Lys Glu
1265                1270


<210> SEQ ID NO 7
<211> LENGTH: 1297
<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum Serotype G
<220> FEATURE:
<221> NAME/KEY: DOMAIN
<222> LOCATION: (1)...(442)
<223> OTHER INFORMATION: Light chain comprising the enzymatic domain.
<221> NAME/KEY: DOMAIN
<222> LOCATION: (443)...(852)
<223> OTHER INFORMATION: Amino-terminal half of heavy chain comprising
      the translocation domain.
<221> NAME/KEY: DOMAIN
<222> LOCATION: (853)...(1297)
<223> OTHER INFORMATION: Carboxyl-terminal half of heavy chain
      comprising the binding domain.

<400> SEQUENCE: 7

Met Pro Val Asn Ile Lys Asn Phe Asn Tyr Asn Asp Pro Ile Asn Asn
 1               5                  10                  15

Asp Asp Ile Ile Met Met Glu Pro Phe Asn Asp Pro Gly Pro Gly Thr
            20                  25                  30

Tyr Tyr Lys Ala Phe Arg Ile Ile Asp Arg Ile Trp Ile Val Pro Glu
        35                  40                  45

Arg Phe Thr Tyr Gly Phe Gln Pro Asp Gln Phe Asn Ala Ser Thr Gly
    50                  55                  60

Val Phe Ser Lys Asp Val Tyr Glu Tyr Tyr Asp Pro Thr Tyr Leu Lys
65                  70                  75                  80

Thr Asp Ala Glu Lys Asp Lys Phe Leu Lys Thr Met Ile Lys Leu Phe
                85                  90                  95
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Asn Arg Ile Asn Ser Lys Pro Ser Gly Gln Arg Leu Leu Asp Met Ile
            100                 105                 110

Val Asp Ala Ile Pro Tyr Leu Gly Asn Ala Ser Thr Pro Pro Asp Lys
        115                 120                 125

Phe Ala Ala Asn Val Ala Asn Val Ser Ile Asn Lys Lys Ile Ile Gln
    130                 135                 140

Pro Gly Ala Glu Asp Gln Ile Lys Gly Leu Met Thr Asn Leu Ile Ile
145                 150                 155                 160

Phe Gly Pro Gly Pro Val Leu Ser Asp Asn Phe Thr Asp Ser Met Ile
                165                 170                 175

Met Asn Gly His Ser Pro Ile Ser Glu Gly Phe Gly Ala Arg Met Met
            180                 185                 190

Ile Arg Phe Cys Pro Ser Cys Leu Asn Val Phe Asn Asn Val Gln Glu
        195                 200                 205

Asn Lys Asp Thr Ser Ile Phe Ser Arg Arg Ala Tyr Phe Ala Asp Pro
    210                 215                 220

Ala Leu Thr Leu Met His Glu Leu Ile His Val Leu His Gly Leu Tyr
225                 230                 235                 240

Gly Ile Lys Ile Ser Asn Leu Pro Ile Thr Pro Asn Thr Lys Glu Phe
                245                 250                 255

Phe Met Gln His Ser Asp Pro Val Gln Ala Glu Glu Leu Tyr Thr Phe
            260                 265                 270

Gly Gly His Asp Pro Ser Val Ile Ser Pro Ser Thr Asp Met Asn Ile
        275                 280                 285

Tyr Asn Lys Ala Leu Gln Asn Phe Gln Asp Ile Ala Asn Arg Leu Asn
    290                 295                 300

Ile Val Ser Ser Ala Gln Gly Ser Gly Ile Asp Ile Ser Leu Tyr Lys
305                 310                 315                 320

Gln Ile Tyr Lys Asn Lys Tyr Asp Phe Val Glu Asp Pro Asn Gly Lys
                325                 330                 335

Tyr Ser Val Asp Lys Asp Lys Phe Asp Lys Leu Tyr Lys Ala Leu Met
            340                 345                 350

Phe Gly Phe Thr Glu Thr Asn Leu Ala Gly Glu Tyr Gly Ile Lys Thr
        355                 360                 365

Arg Tyr Ser Tyr Phe Ser Glu Tyr Leu Pro Pro Ile Lys Thr Glu Lys
    370                 375                 380

Leu Leu Asp Asn Thr Ile Tyr Thr Gln Asn Glu Gly Phe Asn Ile Ala
385                 390                 395                 400

Ser Lys Asn Leu Lys Thr Glu Phe Asn Gly Gln Asn Lys Ala Val Asn
                405                 410                 415

Lys Glu Ala Tyr Glu Glu Ile Ser Leu Glu His Leu Val Ile Tyr Arg
            420                 425                 430

Ile Ala Met Cys Lys Pro Val Met Tyr Lys Asn Thr Gly Lys Ser Glu
        435                 440                 445

Gln Cys Ile Ile Val Asn Asn Glu Asp Leu Phe Phe Ile Ala Asn Lys
    450                 455                 460

Asp Ser Phe Ser Lys Asp Leu Ala Lys Ala Glu Thr Ile Ala Tyr Asn
465                 470                 475                 480

Thr Gln Asn Asn Thr Ile Glu Asn Asn Phe Ser Ile Asp Gln Leu Ile
                485                 490                 495

Leu Asp Asn Asp Leu Ser Ser Gly Ile Asp Leu Pro Asn Glu Asn Thr
            500                 505                 510
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Glu Pro Phe Thr Asn Phe Asp Asp Ile Asp Ile Pro Val Tyr Ile Lys
        515                 520                 525

Gln Ser Ala Leu Lys Lys Ile Phe Val Asp Gly Asp Ser Leu Phe Glu
    530                 535                 540

Tyr Leu His Ala Gln Thr Phe Pro Ser Asn Ile Glu Asn Leu Gln Leu
545                 550                 555                 560

Thr Asn Ser Leu Asn Asp Ala Leu Arg Asn Asn Asn Lys Val Tyr Thr
                565                 570                 575

Phe Phe Ser Thr Asn Leu Val Glu Lys Ala Asn Thr Val Val Gly Ala
            580                 585                 590

Ser Leu Phe Val Asn Trp Val Lys Gly Val Ile Asp Asp Phe Thr Ser
        595                 600                 605

Glu Ser Thr Gln Lys Ser Thr Ile Asp Lys Val Ser Asp Val Ser Ile
    610                 615                 620

Ile Ile Pro Tyr Ile Gly Pro Ala Leu Asn Val Gly Asn Glu Thr Ala
625                 630                 635                 640

Lys Glu Asn Phe Lys Asn Ala Phe Glu Ile Gly Gly Ala Ala Ile Leu
                645                 650                 655

Met Glu Phe Ile Pro Glu Leu Ile Val Pro Ile Val Gly Phe Phe Thr
            660                 665                 670

Leu Glu Ser Tyr Val Gly Asn Lys Gly His Ile Ile Met Thr Ile Ser
        675                 680                 685

Asn Ala Leu Lys Lys Arg Asp Gln Lys Trp Thr Asp Met Tyr Gly Leu
    690                 695                 700

Ile Val Ser Gln Trp Leu Ser Thr Val Asn Thr Gln Phe Tyr Thr Ile
705                 710                 715                 720

Lys Glu Arg Met Tyr Asn Ala Leu Asn Asn Gln Ser Gln Ala Ile Glu
                725                 730                 735

Lys Ile Ile Glu Asp Gln Tyr Asn Arg Tyr Ser Glu Glu Asp Lys Met
            740                 745                 750

Asn Ile Asn Ile Asp Phe Asn Asp Ile Asp Phe Lys Leu Asn Gln Ser
        755                 760                 765

Ile Asn Leu Ala Ile Asn Asn Ile Asp Asp Phe Ile Asn Gln Cys Ser
    770                 775                 780

Ile Ser Tyr Leu Met Asn Arg Met Ile Pro Leu Ala Val Lys Lys Leu
785                 790                 795                 800

Lys Asp Phe Asp Asp Asn Leu Lys Arg Asp Leu Leu Glu Tyr Ile Asp
                805                 810                 815

Thr Asn Glu Leu Tyr Leu Leu Asp Glu Val Asn Ile Leu Lys Ser Lys
            820                 825                 830

Val Asn Arg His Leu Lys Asp Ser Ile Pro Phe Asp Leu Ser Leu Tyr
        835                 840                 845

Thr Lys Asp Thr Ile Leu Ile Gln Val Phe Asn Asn Tyr Ile Ser Asn
    850                 855                 860

Ile Ser Ser Asn Ala Ile Leu Ser Leu Ser Tyr Arg Gly Gly Arg Leu
865                 870                 875                 880

Ile Asp Ser Ser Gly Tyr Gly Ala Thr Met Asn Val Gly Ser Asp Val
                885                 890                 895

Ile Phe Asn Asp Ile Gly Asn Gly Gln Phe Lys Leu Asn Asn Ser Glu
            900                 905                 910

Asn Ser Asn Ile Thr Ala His Gln Ser Lys Phe Val Val Tyr Asp Ser
        915                 920                 925

Met Phe Asp Asn Phe Ser Ile Asn Phe Trp Val Arg Thr Pro Lys Tyr
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    930                 935                 940

Asn Asn Asn Asp Ile Gln Thr Tyr Leu Gln Asn Glu Tyr Thr Ile Ile
945                 950                 955                 960

Ser Cys Ile Lys Asn Asp Ser Gly Trp Lys Val Ser Ile Lys Gly Asn
                965                 970                 975

Arg Ile Ile Trp Thr Leu Ile Asp Val Asn Ala Lys Ser Lys Ser Ile
            980                 985                 990

Phe Phe Glu Tyr Ser Ile Lys Asp Asn Ile Ser Asp Tyr Ile Asn Lys
        995                 1000                1005

Trp Phe Ser Ile Thr Ile Thr Asn Asp Arg Leu Gly Asn Ala Asn Ile
    1010                1015                1020

Tyr Ile Asn Gly Ser Leu Lys Lys Ser Glu Lys Ile Leu Asn Leu Asp
1025                1030                1035                1040

Arg Ile Asn Ser Ser Asn Asp Ile Asp Phe Lys Leu Ile Asn Cys Thr
                1045                1050                1055

Asp Thr Thr Lys Phe Val Trp Ile Lys Asp Phe Asn Ile Phe Gly Arg
            1060                1065                1070

Glu Leu Asn Ala Thr Glu Val Ser Ser Leu Tyr Trp Ile Gln Ser Ser
        1075                1080                1085

Thr Asn Thr Leu Lys Asp Phe Trp Gly Asn Pro Leu Arg Tyr Asp Thr
    1090                1095                1100

Gln Tyr Tyr Leu Phe Asn Gln Gly Met Gln Asn Ile Tyr Ile Lys Tyr
1105                1110                1115                1120

Phe Ser Lys Ala Ser Met Gly Glu Thr Ala Pro Arg Thr Asn Phe Asn
                1125                1130                1135

Asn Ala Ala Ile Asn Tyr Gln Asn Leu Tyr Leu Gly Leu Arg Phe Ile
            1140                1145                1150

Ile Lys Lys Ala Ser Asn Ser Arg Asn Ile Asn Asn Asp Asn Ile Val
        1155                1160                1165

Arg Glu Gly Asp Tyr Ile Tyr Leu Asn Ile Asp Asn Ile Ser Asp Glu
    1170                1175                1180

Ser Tyr Arg Val Tyr Val Leu Val Asn Ser Lys Glu Ile Gln Thr Gln
1185                1190                1195                1200

Leu Phe Leu Ala Pro Ile Asn Asp Asp Pro Thr Phe Tyr Asp Val Leu
                1205                1210                1215

Gln Ile Lys Lys Tyr Tyr Glu Lys Thr Thr Tyr Asn Cys Gln Ile Leu
            1220                1225                1230

Cys Glu Lys Asp Thr Lys Thr Phe Gly Leu Phe Gly Ile Gly Lys Phe
        1235                1240                1245

Val Lys Asp Tyr Gly Tyr Val Trp Asp Thr Tyr Asp Asn Tyr Phe Cys
    1250                1255                1260

Ile Ser Gln Trp Tyr Leu Arg Arg Ile Ser Glu Asn Ile Asn Lys Leu
1265                1270                1275                1280

Arg Leu Gly Cys Asn Trp Gln Phe Ile Pro Val Asp Glu Gly Trp Thr
                1285                1290                1295

Glu
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<210> SEQ ID NO 8
<211> LENGTH: 1315
<212> TYPE: PRT
<213> ORGANISM: Clostridium tetani
<220> FEATURE:
<221> NAME/KEY: DOMAIN
<222> LOCATION: (1)...(441)
<223> OTHER INFORMATION: Light chain comprising the enzymatic domain.

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<221> NAME/KEY: DOMAIN
<222> LOCATION: (442)...(870)
<223> OTHER INFORMATION: Amino-terminal half of heavy chain comprising the translocation domain.
<221> NAME/KEY: DOMAIN
<222> LOCATION: (871)...(1315)
<223> OTHER INFORMATION: Carboxyl-terminal half of heavy chain comprising the binding domain.

<400> SEQUENCE: 8

Met Pro Ile Thr Ile Asn Asn Phe Arg Tyr Ser Asp Pro Val Asn Asn
1 5 10 15

Asp Thr Ile Ile Met Met Glu Pro Pro Tyr Cys Lys Gly Leu Asp Ile
20 25 30

Tyr Tyr Lys Ala Phe Lys Ile Thr Asp Arg Ile Trp Ile Val Pro Glu
35 40 45

Arg Tyr Glu Phe Gly Thr Lys Pro Glu Asp Phe Asn Pro Pro Ser Ser
50 55 60

Leu Ile Glu Gly Ala Ser Glu Tyr Tyr Asp Pro Asn Tyr Leu Arg Thr
65 70 75 80

Asp Ser Asp Lys Asp Arg Phe Leu Gln Thr Met Val Lys Leu Phe Asn
85 90 95

Arg Ile Lys Asn Asn Val Ala Gly Glu Ala Leu Leu Asp Lys Ile Ile
100 105 110

Asn Ala Ile Pro Tyr Leu Gly Asn Ser Tyr Ser Leu Leu Asp Lys Phe
115 120 125

Asp Thr Asn Ser Asn Ser Val Ser Phe Asn Leu Leu Glu Gln Asp Pro
130 135 140

Ser Gly Ala Thr Thr Lys Ser Ala Met Leu Thr Asn Leu Ile Ile Phe
145 150 155 160

Gly Pro Gly Pro Val Leu Asn Lys Asn Glu Val Arg Gly Ile Val Leu
165 170 175

Arg Val Asp Asn Lys Asn Tyr Phe Pro Cys Arg Asp Gly Phe Gly Ser
180 185 190

Ile Met Gln Met Ala Phe Cys Pro Glu Tyr Val Pro Thr Phe Asp Asn
195 200 205

Val Ile Glu Asn Ile Thr Ser Leu Thr Ile Gly Lys Ser Lys Tyr Phe
210 215 220

Gln Asp Pro Ala Leu Leu Leu Met His Glu Leu Ile His Val Leu His
225 230 235 240

Gly Leu Tyr Gly Met Gln Val Ser Ser His Glu Ile Ile Pro Ser Lys
245 250 255

Gln Glu Ile Tyr Met Gln His Thr Tyr Pro Ile Ser Ala Glu Glu Leu
260 265 270

Phe Thr Phe Gly Gly Gln Asp Ala Asn Leu Ile Ser Ile Asp Ile Lys
275 280 285

Asn Asp Leu Tyr Glu Lys Thr Leu Asn Asp Tyr Lys Ala Ile Ala Asn
290 295 300

Lys Leu Ser Gln Val Thr Ser Cys Asn Asp Pro Asn Ile Asp Ile Asp
305 310 315 320

Ser Tyr Lys Gln Ile Tyr Gln Gln Lys Tyr Gln Phe Asp Lys Asp Ser
325 330 335

Asn Gly Gln Tyr Ile Val Asn Glu Asp Lys Phe Gln Ile Leu Tyr Asn
340 345 350

Ser Ile Met Tyr Gly Phe Thr Glu Ile Glu Leu Gly Lys Lys Phe Asn
355 360 365

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Ile Lys Thr Arg Leu Ser Tyr Phe Ser Met Asn His Asp Pro Val Lys
    370                 375                 380

Ile Pro Asn Leu Leu Asp Asp Thr Ile Tyr Asn Asp Thr Glu Gly Phe
385                 390                 395                 400

Asn Ile Glu Ser Lys Asp Leu Lys Ser Glu Tyr Lys Gly Gln Asn Met
                405                 410                 415

Arg Val Asn Thr Asn Ala Phe Arg Asn Val Asp Gly Ser Gly Leu Val
            420                 425                 430

Ser Lys Leu Ile Gly Leu Cys Lys Lys Ile Ile Pro Pro Thr Asn Ile
        435                 440                 445

Arg Glu Asn Leu Tyr Asn Arg Thr Ala Ser Leu Thr Asp Leu Gly Gly
    450                 455                 460

Glu Leu Cys Ile Lys Ile Lys Asn Glu Asp Leu Thr Phe Ile Ala Glu
465                 470                 475                 480

Lys Asn Ser Phe Ser Glu Glu Pro Phe Gln Asp Glu Ile Val Ser Tyr
                485                 490                 495

Asn Thr Lys Asn Lys Pro Leu Asn Phe Asn Tyr Ser Leu Asp Lys Ile
            500                 505                 510

Ile Val Asp Tyr Asn Leu Gln Ser Lys Ile Thr Leu Pro Asn Asp Arg
        515                 520                 525

Thr Thr Pro Val Thr Lys Gly Ile Pro Tyr Ala Pro Glu Tyr Lys Ser
    530                 535                 540

Asn Ala Ala Ser Thr Ile Glu Ile His Asn Ile Asp Asp Asn Thr Ile
545                 550                 555                 560

Tyr Gln Tyr Leu Tyr Ala Gln Lys Ser Pro Thr Thr Leu Gln Arg Ile
                565                 570                 575

Thr Met Thr Asn Ser Val Asp Asp Ala Leu Ile Asn Ser Thr Lys Ile
            580                 585                 590

Tyr Ser Tyr Phe Pro Ser Val Ile Ser Lys Val Asn Gln Gly Ala Gln
        595                 600                 605

Gly Ile Leu Phe Leu Gln Trp Val Arg Asp Ile Ile Asp Asp Phe Thr
    610                 615                 620

Asn Glu Ser Ser Gln Lys Thr Thr Ile Asp Lys Ile Ser Asp Val Ser
625                 630                 635                 640

Thr Ile Val Pro Tyr Ile Gly Pro Ala Leu Asn Ile Val Lys Gln Gly
                645                 650                 655

Tyr Glu Gly Asn Phe Ile Gly Ala Leu Glu Thr Thr Gly Val Val Leu
            660                 665                 670

Leu Leu Glu Tyr Ile Pro Glu Ile Thr Leu Pro Val Ile Ala Ala Leu
        675                 680                 685

Ser Ile Ala Glu Ser Ser Thr Gln Lys Glu Lys Ile Ile Lys Thr Ile
    690                 695                 700

Asp Asn Phe Leu Glu Lys Arg Tyr Glu Lys Trp Ile Glu Val Tyr Lys
705                 710                 715                 720

Leu Val Lys Ala Lys Trp Leu Gly Thr Val Asn Thr Gln Phe Gln Lys
                725                 730                 735

Arg Ser Tyr Gln Met Tyr Arg Ser Leu Glu Tyr Gln Val Asp Ala Ile
            740                 745                 750

Lys Lys Ile Ile Asp Tyr Glu Tyr Lys Ile Tyr Ser Gly Pro Asp Lys
        755                 760                 765

Glu Gln Ile Ala Asp Glu Ile Asn Asn Leu Lys Asn Lys Leu Glu Glu
    770                 775                 780
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Lys Ala Asn Lys Ala Met Ile Asn Ile Asn Ile Phe Met Arg Glu Ser
785                 790                 795                 800

Ser Arg Ser Phe Leu Val Asn Gln Met Ile Asn Glu Ala Lys Lys Gln
                805                 810                 815

Leu Leu Glu Phe Asp Thr Gln Ser Lys Asn Ile Leu Met Gln Tyr Ile
            820                 825                 830

Lys Ala Asn Ser Lys Phe Ile Gly Ile Thr Glu Leu Lys Lys Leu Glu
        835                 840                 845

Ser Lys Ile Asn Lys Val Phe Ser Thr Pro Ile Pro Phe Ser Tyr Ser
    850                 855                 860

Lys Asn Leu Asp Cys Trp Val Asp Asn Glu Glu Asp Ile Asp Val Ile
865                 870                 875                 880

Leu Lys Lys Ser Thr Ile Leu Asn Leu Asp Ile Asn Asn Asp Ile Ile
                885                 890                 895

Ser Asp Ile Ser Gly Phe Asn Ser Ser Val Ile Thr Tyr Pro Asp Ala
            900                 905                 910

Gln Leu Val Pro Gly Ile Asn Gly Lys Ala Ile His Leu Val Asn Asn
        915                 920                 925

Glu Ser Ser Glu Val Ile Val His Lys Ala Met Asp Ile Glu Tyr Asn
    930                 935                 940

Asp Met Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg Val Pro Lys
945                 950                 955                 960

Val Ser Ala Ser His Leu Glu Gln Tyr Gly Thr Asn Glu Tyr Ser Ile
                965                 970                 975

Ile Ser Ser Met Lys Lys His Ser Leu Ser Ile Gly Ser Gly Trp Ser
            980                 985                 990

Val Ser Leu Lys Gly Asn Asn Leu Ile Trp Thr Leu Lys Asp Ser Ala
        995                 1000                1005

Gly Glu Val Arg Gln Ile Thr Phe Arg Asp Leu Pro Asp Lys Phe Asn
    1010                1015                1020

Ala Tyr Leu Ala Asn Lys Trp Val Phe Ile Thr Ile Thr Asn Asp Arg
1025                1030                1035                1040

Leu Ser Ser Ala Asn Leu Tyr Ile Asn Gly Val Leu Met Gly Ser Ala
                1045                1050                1055

Glu Ile Thr Gly Leu Gly Ala Ile Arg Glu Asp Asn Asn Ile Thr Leu
            1060                1065                1070

Lys Leu Asp Arg Cys Asn Asn Asn Asn Gln Tyr Val Ser Ile Asp Lys
        1075                1080                1085

Phe Arg Ile Phe Cys Lys Ala Leu Asn Pro Lys Glu Ile Glu Lys Leu
    1090                1095                1100

Tyr Thr Ser Tyr Leu Ser Ile Thr Phe Leu Arg Asp Phe Trp Gly Asn
1105                1110                1115                1120

Pro Leu Arg Tyr Asp Thr Glu Tyr Tyr Leu Ile Pro Val Ala Ser Ser
                1125                1130                1135

Ser Lys Asp Val Gln Leu Lys Asn Ile Thr Asp Tyr Met Tyr Leu Thr
            1140                1145                1150

Asn Ala Pro Ser Tyr Thr Asn Gly Lys Leu Asn Ile Tyr Tyr Arg Arg
        1155                1160                1165

Leu Tyr Asn Gly Leu Lys Phe Ile Ile Lys Arg Tyr Thr Pro Asn Asn
    1170                1175                1180

Glu Ile Asp Ser Phe Val Lys Ser Gly Asp Phe Ile Lys Leu Tyr Val
1185                1190                1195                1200

Ser Tyr Asn Asn Asn Glu His Ile Val Gly Tyr Pro Lys Asp Gly Asn
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                1205                1210                1215

Ala Phe Asn Asn Leu Asp Arg Ile Leu Arg Val Gly Tyr Asn Ala Pro
            1220                1225                1230

Gly Ile Pro Leu Tyr Lys Lys Met Glu Ala Val Lys Leu Arg Asp Leu
        1235                1240                1245

Lys Thr Tyr Ser Val Gln Leu Lys Leu Tyr Asp Asp Lys Asn Ala Ser
    1250                1255                1260

Leu Gly Leu Val Gly Thr His Asn Gly Gln Ile Gly Asn Asp Pro Asn
1265                1270                1275                1280

Arg Asp Ile Leu Ile Ala Ser Asn Trp Tyr Phe Asn His Leu Lys Asp
                1285                1290                1295

Lys Ile Leu Gly Cys Asp Trp Tyr Phe Val Pro Thr Asp Glu Gly Trp
            1300                1305                1310

Thr Asn Asp
        1315


<210> SEQ ID NO 9
<211> LENGTH: 1268
<212> TYPE: PRT
<213> ORGANISM: Clostridium baratii

<400> SEQUENCE: 9

Met Pro Val Asn Ile Asn Asn Phe Asn Tyr Asn Asp Pro Ile Asn Asn
 1               5                  10                  15

Thr Thr Ile Leu Tyr Met Lys Met Pro Tyr Tyr Glu Asp Ser Asn Lys
            20                  25                  30

Tyr Tyr Lys Ala Phe Glu Ile Met Asp Asn Val Trp Ile Ile Pro Glu
        35                  40                  45

Arg Asn Ile Ile Gly Lys Lys Pro Ser Asp Phe Tyr Pro Pro Ile Ser
    50                  55                  60

Leu Asp Ser Gly Ser Ser Ala Tyr Tyr Asp Pro Asn Tyr Leu Thr Thr
65                  70                  75                  80

Asp Ala Glu Lys Asp Arg Phe Leu Lys Thr Val Ile Lys Leu Phe Asn
                85                  90                  95

Arg Ile Asn Ser Asn Pro Ala Gly Gln Val Leu Leu Glu Glu Ile Lys
            100                 105                 110

Asn Gly Lys Pro Tyr Leu Gly Asn Asp His Thr Ala Val Asn Glu Phe
        115                 120                 125

Cys Ala Asn Asn Arg Ser Thr Ser Val Glu Ile Lys Glu Ser Asn Gly
    130                 135                 140

Thr Thr Asp Ser Met Leu Leu Asn Leu Val Ile Leu Gly Pro Gly Pro
145                 150                 155                 160

Asn Ile Leu Glu Cys Ser Thr Phe Pro Val Arg Ile Phe Pro Asn Asn
                165                 170                 175

Ile Ala Tyr Asp Pro Ser Glu Lys Gly Phe Gly Ser Ile Gln Leu Met
            180                 185                 190

Ser Phe Ser Thr Glu Tyr Glu Tyr Ala Phe Asn Asp Asn Thr Asp Leu
        195                 200                 205

Phe Ile Ala Asp Pro Ala Ile Ser Leu Ala His Glu Leu Ile His Val
    210                 215                 220

Leu His Gly Leu Tyr Gly Ala Lys Gly Val Thr Asn Lys Lys Val Ile
225                 230                 235                 240

Glu Val Asp Gln Gly Ala Leu Met Ala Ala Glu Lys Asp Ile Lys Ile
                245                 250                 255
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Glu Glu Phe Ile Thr Phe Gly Gly Gln Asp Leu Asn Ile Ile Thr Asn
            260                 265                 270

Ser Thr Asn Gln Lys Ile Tyr Val Ile Leu Leu Ser Asn Tyr Thr Ala
        275                 280                 285

Ile Ala Ser Arg Leu Ser Gln Val Asn Arg Asn Asn Ser Ala Leu Asn
    290                 295                 300

Thr Thr Tyr Tyr Lys Asn Phe Phe Gln Trp Lys Tyr Gly Leu Asp Gln
305                 310                 315                 320

Asp Ser Asn Gly Asn Tyr Thr Val Asn Ile Ser Lys Phe Asn Ala Ile
                325                 330                 335

Tyr Lys Lys Leu Phe Ser Phe Thr Glu Cys Asp Leu Ala Gln Lys Phe
            340                 345                 350

Gln Val Lys Asn Arg Ser Asn Tyr Leu Phe His Phe Lys Pro Phe Arg
        355                 360                 365

Leu Leu Asp Leu Leu Asp Asp Asn Ile Tyr Ser Ile Ser Glu Gly Phe
    370                 375                 380

Asn Ile Gly Ser Leu Arg Val Asn Asn Asn Gly Gln Asn Ile Asn Leu
385                 390                 395                 400

Asn Ser Arg Ile Val Gly Pro Ile Pro Asp Asn Gly Leu Val Glu Arg
                405                 410                 415

Phe Val Gly Leu Cys Lys Ser Ile Val Ser Lys Lys Gly Thr Lys Asn
            420                 425                 430

Ser Leu Cys Ile Lys Val Asn Asn Arg Asp Leu Phe Phe Val Ala Ser
        435                 440                 445

Glu Ser Ser Tyr Asn Glu Asn Gly Ile Asn Ser Pro Lys Glu Ile Asp
    450                 455                 460

Asp Thr Thr Ile Thr Asn Asn Asn Tyr Lys Lys Asn Leu Asp Glu Val
465                 470                 475                 480

Ile Leu Asp Tyr Asn Ser Asp Ala Ile Pro Asn Leu Ser Ser Arg Leu
                485                 490                 495

Leu Asn Thr Thr Ala Gln Asn Asp Ser Tyr Val Pro Lys Tyr Asp Ser
            500                 505                 510

Asn Gly Thr Ser Glu Ile Lys Glu Tyr Thr Val Asp Lys Leu Asn Val
        515                 520                 525

Phe Phe Tyr Leu Tyr Ala Gln Lys Ala Pro Glu Gly Glu Ser Ala Ile
    530                 535                 540

Ser Leu Thr Ser Ser Val Asn Thr Ala Leu Leu Asp Ala Ser Lys Val
545                 550                 555                 560

Tyr Thr Phe Phe Ser Ser Asp Phe Ile Asn Thr Val Asn Lys Pro Val
                565                 570                 575

Gln Ala Ala Leu Phe Ile Ser Trp Ile Gln Gln Val Ile Asn Asp Phe
            580                 585                 590

Thr Thr Glu Ala Thr Gln Lys Ser Thr Ile Asp Lys Ile Ala Asp Ile
        595                 600                 605

Ser Leu Ile Val Pro Tyr Val Gly Leu Ala Leu Asn Ile Gly Asn Glu
    610                 615                 620

Val Gln Lys Gly Asn Phe Lys Glu Ala Ile Glu Leu Leu Gly Ala Gly
625                 630                 635                 640

Ile Leu Leu Glu Phe Val Pro Glu Leu Leu Ile Pro Thr Ile Leu Val
                645                 650                 655

Phe Thr Ile Lys Ser Phe Ile Asn Ser Asp Asp Ser Lys Asn Lys Ile
            660                 665                 670

Ile Lys Ala Ile Asn Asn Ala Leu Arg Glu Arg Glu Leu Lys Trp Lys
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675 680 685

Glu Val Tyr Ser Trp Ile Val Ser Asn Trp Leu Thr Arg Ile Asn Thr
690 695 700

Gln Phe Asn Lys Arg Lys Glu Gln Met Tyr Gln Ala Leu Gln Asn Gln
705 710 715 720

Val Asp Gly Ile Lys Lys Ile Ile Glu Tyr Lys Tyr Asn Asn Tyr Thr
725 730 735

Leu Asp Glu Lys Asn Arg Leu Arg Ala Glu Tyr Asn Ile Tyr Ser Ile
740 745 750

Lys Glu Glu Leu Asn Lys Lys Val Ser Leu Ala Met Gln Asn Ile Asp
755 760 765

Arg Phe Leu Thr Glu Ser Ser Ile Ser Tyr Leu Met Lys Leu Ile Asn
770 775 780

Glu Ala Lys Ile Asn Lys Leu Ser Glu Tyr Asp Lys Arg Val Asn Gln
785 790 795 800

Tyr Leu Leu Asn Tyr Ile Leu Glu Asn Ser Ser Thr Leu Gly Thr Ser
805 810 815

Ser Val Pro Glu Leu Asn Asn Leu Val Ser Asn Thr Leu Asn Asn Ser
820 825 830

Ile Pro Phe Glu Leu Ser Glu Tyr Thr Asn Asp Lys Ile Leu Ile His
835 840 845

Ile Leu Ile Arg Phe Tyr Lys Arg Ile Ile Asp Ser Ser Ile Leu Asn
850 855 860

Met Lys Tyr Glu Asn Asn Arg Phe Ile Asp Ser Ser Gly Tyr Gly Ser
865 870 875 880

Asn Ile Ser Ile Asn Gly Asp Ile Tyr Ile Tyr Ser Thr Asn Arg Asn
885 890 895

Gln Phe Gly Ile Tyr Ser Ser Arg Leu Ser Glu Val Asn Ile Thr Gln
900 905 910

Asn Asn Thr Ile Ile Tyr Asn Ser Arg Tyr Gln Asn Phe Ser Val Ser
915 920 925

Phe Trp Val Arg Ile Pro Lys Tyr Asn Asn Leu Lys Asn Leu Asn Asn
930 935 940

Glu Tyr Thr Ile Ile Asn Cys Met Arg Asn Asn Asn Ser Gly Trp Lys
945 950 955 960

Ile Ser Leu Asn Tyr Asn Asn Ile Ile Trp Thr Leu Gln Asp Thr Thr
965 970 975

Gly Asn Asn Gln Lys Leu Val Phe Asn Tyr Thr Gln Met Ile Asp Ile
980 985 990

Ser Asp Tyr Ile Asn Lys Trp Thr Phe Val Thr Ile Thr Asn Asn Arg
995 1000 1005

Leu Gly His Ser Lys Leu Tyr Ile Asn Gly Asn Leu Thr Asp Gln Lys
1010 1015 1020

Ser Ile Leu Asn Leu Gly Asn Ile His Val Asp Asp Asn Ile Leu Phe
1025 1030 1035 1040

Lys Ile Val Gly Cys Asn Asp Thr Arg Tyr Val Gly Ile Arg Tyr Phe
1045 1050 1055

Lys Ile Phe Asn Met Glu Leu Asp Lys Thr Glu Ile Glu Thr Leu Tyr
1060 1065 1070

His Ser Glu Pro Asp Ser Thr Ile Leu Lys Asp Phe Trp Gly Asn Tyr
1075 1080 1085

Leu Leu Tyr Asn Lys Lys Tyr Tyr Leu Leu Asn Leu Leu Lys Pro Asn
1090 1095 1100

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Met Ser Val Thr Lys Asn Ser Asp Ile Leu Asn Ile Asn Arg Gln Arg
1105 1110 1115 1120

Gly Ile Tyr Ser Lys Thr Asn Ile Phe Ser Asn Ala Arg Leu Tyr Thr
1125 1130 1135

Gly Val Glu Val Ile Ile Arg Lys Val Gly Ser Thr Asp Thr Ser Asn
1140 1145 1150

Thr Asp Asn Phe Val Arg Lys Asn Asp Thr Val Tyr Ile Asn Val Val
1155 1160 1165

Asp Gly Asn Ser Glu Tyr Gln Leu Tyr Ala Asp Val Ser Thr Ser Ala
1170 1175 1180

Val Glu Lys Thr Ile Lys Leu Arg Arg Ile Ser Asn Ser Asn Tyr Asn
1185 1190 1195 1200

Ser Asn Gln Met Ile Ile Met Asp Ser Ile Gly Asp Asn Cys Thr Met
1205 1210 1215

Asn Phe Lys Thr Asn Asn Gly Asn Asp Ile Gly Leu Leu Gly Phe His
1220 1225 1230

Leu Asn Asn Leu Val Ala Ser Ser Trp Tyr Tyr Lys Asn Ile Arg Asn
1235 1240 1245

Asn Thr Arg Asn Asn Gly Cys Phe Trp Ser Phe Ile Ser Lys Glu His
1250 1255 1260

Gly Trp Gln Glu
1265

<210> SEQ ID NO 10
<211> LENGTH: 1251
<212> TYPE: PRT
<213> ORGANISM: Clostridium butyricum

<400> SEQUENCE: 10

Met Pro Thr Ile Asn Ser Phe Asn Tyr Asn Asp Pro Val Asn Asn Arg
1 5 10 15

Thr Ile Leu Tyr Ile Lys Pro Gly Gly Cys Gln Gln Phe Tyr Lys Ser
20 25 30

Phe Asn Ile Met Lys Asn Ile Trp Ile Ile Pro Glu Arg Asn Val Ile
35 40 45

Gly Thr Ile Pro Gln Asp Phe Leu Pro Pro Thr Ser Leu Lys Asn Gly
50 55 60

Asp Ser Ser Tyr Tyr Asp Pro Asn Tyr Leu Gln Ser Asp Gln Glu Lys
65 70 75 80

Asp Lys Phe Leu Lys Ile Val Thr Lys Ile Phe Asn Arg Ile Asn Asp
85 90 95

Asn Leu Ser Gly Arg Ile Leu Leu Glu Glu Leu Ser Lys Ala Asn Pro
100 105 110

Tyr Leu Gly Asn Asp Asn Thr Pro Asp Gly Asp Phe Ile Ile Asn Asp
115 120 125

Ala Ser Ala Val Pro Ile Gln Phe Ser Asn Gly Ser Gln Ser Ile Leu
130 135 140

Leu Pro Asn Val Ile Ile Met Gly Ala Glu Pro Asp Leu Phe Glu Thr
145 150 155 160

Asn Ser Ser Asn Ile Ser Leu Arg Asn Asn Tyr Met Pro Ser Asn His
165 170 175

Gly Phe Gly Ser Ile Ala Ile Val Thr Phe Ser Pro Glu Tyr Ser Phe
180 185 190

Arg Phe Lys Asp Asn Ser Met Asn Glu Phe Ile Gln Asp Pro Ala Leu

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        195                 200                 205

Thr Leu Met His Glu Leu Ile His Ser Leu His Gly Leu Tyr Gly Ala
    210                 215                 220

Lys Gly Ile Thr Thr Lys Tyr Thr Ile Thr Gln Lys Gln Asn Pro Leu
225                 230                 235                 240

Ile Thr Asn Ile Arg Gly Thr Asn Ile Glu Glu Phe Leu Thr Phe Gly
                245                 250                 255

Gly Thr Asp Leu Asn Ile Ile Thr Ser Ala Gln Ser Asn Asp Ile Tyr
            260                 265                 270

Thr Asn Leu Leu Ala Asp Tyr Lys Lys Ile Ala Ser Lys Leu Ser Lys
        275                 280                 285

Val Gln Val Ser Asn Pro Leu Leu Asn Pro Tyr Lys Asp Val Phe Glu
    290                 295                 300

Ala Lys Tyr Gly Leu Asp Lys Asp Ala Ser Gly Ile Tyr Ser Val Asn
305                 310                 315                 320

Ile Asn Lys Phe Asn Asp Ile Phe Lys Lys Leu Tyr Ser Phe Thr Glu
                325                 330                 335

Phe Asp Leu Ala Thr Lys Phe Gln Val Lys Cys Arg Gln Thr Tyr Ile
            340                 345                 350

Gly Gln Tyr Lys Tyr Phe Lys Leu Ser Asn Leu Leu Asn Asp Ser Ile
        355                 360                 365

Tyr Asn Ile Ser Glu Gly Tyr Asn Ile Asn Asn Leu Lys Val Asn Phe
    370                 375                 380

Arg Gly Gln Asn Ala Asn Leu Asn Pro Arg Ile Ile Thr Pro Ile Thr
385                 390                 395                 400

Gly Arg Gly Leu Val Lys Lys Ile Ile Arg Phe Cys Lys Asn Ile Val
                405                 410                 415

Ser Val Lys Gly Ile Arg Lys Ser Ile Cys Ile Glu Ile Asn Asn Gly
            420                 425                 430

Glu Leu Phe Phe Val Ala Ser Glu Asn Ser Tyr Asn Asp Asp Asn Ile
        435                 440                 445

Asn Thr Pro Lys Glu Ile Asp Asp Thr Val Thr Ser Asn Asn Asn Tyr
    450                 455                 460

Glu Asn Asp Leu Asp Gln Val Ile Leu Asn Phe Asn Ser Glu Ser Ala
465                 470                 475                 480

Pro Gly Leu Ser Asp Glu Lys Leu Asn Leu Thr Ile Gln Asn Asp Ala
                485                 490                 495

Tyr Ile Pro Lys Tyr Asp Ser Asn Gly Thr Ser Asp Ile Glu Gln His
            500                 505                 510

Asp Val Asn Glu Leu Asn Val Phe Phe Tyr Leu Asp Ala Gln Lys Val
        515                 520                 525

Pro Glu Gly Glu Asn Asn Val Asn Leu Thr Ser Ser Ile Asp Thr Ala
    530                 535                 540

Leu Leu Glu Gln Pro Lys Ile Tyr Thr Phe Phe Ser Ser Glu Phe Ile
545                 550                 555                 560

Asn Asn Val Asn Lys Pro Val Gln Ala Ala Leu Phe Val Gly Trp Ile
                565                 570                 575

Gln Gln Val Leu Val Asp Phe Thr Thr Glu Ala Asn Gln Lys Ser Thr
            580                 585                 590

Val Asp Lys Ile Ala Asp Ile Ser Ile Val Val Pro Tyr Ile Gly Leu
        595                 600                 605

Ala Leu Asn Ile Gly Asn Glu Ala Gln Lys Gly Asn Phe Lys Asp Ala
    610                 615                 620
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Leu Glu Leu Leu Gly Ala Gly Ile Leu Leu Glu Phe Glu Pro Glu Leu
625                 630                 635                 640

Leu Ile Pro Thr Ile Leu Val Phe Thr Ile Lys Ser Phe Leu Gly Ser
                645                 650                 655

Ser Asp Asn Lys Asn Lys Val Ile Lys Ala Ile Asn Asn Ala Leu Lys
            660                 665                 670

Glu Arg Asp Glu Lys Trp Lys Glu Val Tyr Ser Phe Ile Val Ser Asn
        675                 680                 685

Trp Met Thr Lys Ile Asn Thr Gln Phe Asn Lys Arg Lys Glu Gln Met
    690                 695                 700

Tyr Gln Ala Leu Gln Asn Gln Val Asn Ala Leu Lys Ala Ile Ile Glu
705                 710                 715                 720

Ser Lys Tyr Asn Ser Tyr Thr Leu Glu Glu Lys Asn Glu Leu Thr Asn
                725                 730                 735

Lys Tyr Asp Ile Glu Gln Ile Glu Asn Glu Leu Asn Gln Lys Val Ser
            740                 745                 750

Ile Ala Met Asn Asn Ile Asp Arg Phe Leu Thr Glu Ser Ser Ile Ser
        755                 760                 765

Tyr Leu Met Lys Leu Ile Asn Glu Val Lys Ile Asn Lys Leu Arg Glu
    770                 775                 780

Tyr Asp Glu Asn Val Lys Thr Tyr Leu Leu Asp Tyr Ile Ile Lys His
785                 790                 795                 800

Gly Ser Ile Leu Gly Glu Ser Gln Gln Glu Leu Asn Ser Met Val Ile
                805                 810                 815

Asp Thr Leu Asn Asn Ser Ile Pro Phe Lys Leu Ser Ser Tyr Thr Asp
            820                 825                 830

Asp Lys Ile Leu Ile Ser Tyr Phe Asn Lys Phe Phe Lys Arg Ile Lys
        835                 840                 845

Ser Ser Ser Val Leu Asn Met Arg Tyr Lys Asn Asp Lys Tyr Val Asp
    850                 855                 860

Thr Ser Gly Tyr Asp Ser Asn Ile Asn Ile Asn Gly Asp Val Tyr Lys
865                 870                 875                 880

Tyr Pro Thr Asn Lys Asn Gln Phe Gly Ile Tyr Asn Asp Lys Leu Ser
                885                 890                 895

Glu Val Asn Ile Ser Gln Asn Asp Tyr Ile Ile Tyr Asp Asn Lys Tyr
            900                 905                 910

Lys Asn Phe Ser Ile Ser Phe Trp Val Arg Ile Pro Asn Tyr Asp Asn
        915                 920                 925

Lys Ile Val Asn Val Asn Asn Glu Tyr Thr Ile Ile Asn Cys Met Arg
    930                 935                 940

Asp Asn Asn Ser Gly Trp Lys Val Ser Leu Asn His Asn Glu Ile Ile
945                 950                 955                 960

Trp Thr Leu Gln Asp Asn Ser Gly Ile Asn Gln Lys Leu Ala Phe Asn
                965                 970                 975

Tyr Gly Asn Ala Asn Gly Ile Ser Asp Tyr Ile Asn Lys Trp Ile Phe
            980                 985                 990

Val Thr Ile Thr Asn Asp Arg Leu Gly Asp Ser Lys Leu Tyr Ile Asn
        995                 1000                1005

Gly Asn Leu Ile Asp Lys Lys Ser Ile Leu Asn Leu Gly Asn Ile His
    1010                1015                1020

Val Ser Asp Asn Ile Leu Phe Lys Ile Val Asn Cys Ser Tyr Thr Arg
1025                1030                1035                1040
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Tyr Ile Gly Ile Arg Tyr Phe Asn Ile Phe Asp Lys Glu Leu Asp Glu
1045 1050 1055

Thr Glu Ile Gln Thr Leu Tyr Asn Asn Glu Pro Asn Ala Asn Ile Leu
1060 1065 1070

Lys Asp Phe Trp Gly Asn Tyr Leu Leu Tyr Asp Lys Glu Tyr Tyr Leu
1075 1080 1085

Leu Asn Val Leu Lys Pro Asn Asn Phe Ile Asn Arg Arg Thr Asp Ser
1090 1095 1100

Thr Leu Ser Ile Asn Asn Ile Arg Ser Thr Ile Leu Leu Ala Asn Arg
1105 1110 1115 1120

Leu Tyr Ser Gly Ile Lys Val Lys Ile Gln Arg Val Asn Asn Ser Ser
1125 1130 1135

Thr Asn Asp Asn Leu Val Arg Lys Asn Asp Gln Val Tyr Ile Asn Phe
1140 1145 1150

Val Ala Ser Lys Thr His Leu Leu Pro Leu Tyr Ala Asp Thr Ala Thr
1155 1160 1165

Thr Asn Lys Glu Lys Thr Ile Lys Ile Ser Ser Ser Gly Asn Arg Phe
1170 1175 1180

Asn Gln Val Val Val Met Asn Ser Val Gly Asn Cys Thr Met Asn Phe
1185 1190 1195 1200

Lys Asn Asn Asn Gly Asn Asn Ile Gly Leu Leu Gly Phe Lys Ala Asp
1205 1210 1215

Thr Val Val Ala Ser Thr Trp Tyr Tyr Thr His Met Arg Asp Asn Thr
1220 1225 1230

Asn Ser Asn Gly Phe Phe Trp Asn Phe Ile Ser Glu Glu His Gly Trp
1235 1240 1245

Gln Glu Lys
1250

<210> SEQ ID NO 11
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: DOMAIN
<222> LOCATION: (1)...(25)
<223> OTHER INFORMATION: BoNT/A di-chain loop region

<400> SEQUENCE: 11

Cys Val Arg Gly Ile Ile Thr Ser Lys Thr Lys Ser Leu Asp Lys Gly
1 5 10 15

Tyr Asn Lys Ala Leu Asn Asp Leu Cys
20 25

<210> SEQ ID NO 12
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: DOMAIN
<222> LOCATION: (1)...(10)
<223> OTHER INFORMATION: BoNT/B di-chain loop region

<400> SEQUENCE: 12

Cys Lys Ser Val Lys Ala Pro Gly Ile Cys
1 5 10

<210> SEQ ID NO 13
<211> LENGTH: 17
<212> TYPE: PRT

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: DOMAIN
<222> LOCATION: (1)...(17)
<223> OTHER INFORMATION: BoNT/C1 di-chain loop region

<400> SEQUENCE: 13

Cys His Lys Ala Ile Asp Gly Arg Ser Leu Tyr Asn Lys Thr Leu Asp
 1   5   10   15

Cys

<210> SEQ ID NO 14
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: DOMAIN
<222> LOCATION: (1)...(14)
<223> OTHER INFORMATION: BoNT/D di-chain loop region

<400> SEQUENCE: 14

Cys Leu Arg Leu Thr Lys Asn Ser Arg Asp Asp Ser Thr Cys
 1   5   10

<210> SEQ ID NO 15
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: DOMAIN
<222> LOCATION: (1)...(15)
<223> OTHER INFORMATION: BoNT/E di-chain loop region

<400> SEQUENCE: 15

Cys Lys Asn Ile Val Ser Val Lys Gly Ile Arg Lys Ser Ile Cys
 1   5   10   15

<210> SEQ ID NO 16
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: DOMAIN
<222> LOCATION: (1)...(17)
<223> OTHER INFORMATION: BoNT/F di-chain loop region

<400> SEQUENCE: 16

Cys Lys Ser Val Ile Pro Arg Lys Gly Thr Lys Ala Pro Pro Arg Leu
 1   5   10   15

Cys

<210> SEQ ID NO 17
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: DOMAIN
<222> LOCATION: (1)...(15)
<223> OTHER INFORMATION: BoNT/G di-chain loop region

<400> SEQUENCE: 17

Cys Lys Pro Val Met Tyr Lys Asn Thr Gly Lys Ser Glu Gln Cys
 1   5   10   15

<210> SEQ ID NO 18
<211> LENGTH: 29
<212> TYPE: PRT

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: DOMAIN
<222> LOCATION: (1)...(29)
<223> OTHER INFORMATION: TeNT di-chain loop region

<400> SEQUENCE: 18

Cys Lys Lys Ile Ile Pro Pro Thr Asn Ile Arg Glu Asn Leu Tyr Asn
 1   5   10   15

Arg Thr Ala Ser Leu Thr Asp Leu Gly Gly Glu Leu Cys
            20   25

<210> SEQ ID NO 19
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: DOMAIN
<222> LOCATION: (1)...(15)
<223> OTHER INFORMATION: BaNT di-chain loop region

<400> SEQUENCE: 19

Cys Lys Ser Ile Val Ser Lys Lys Gly Thr Lys Asn Ser Leu Cys
 1   5   10   15

<210> SEQ ID NO 20
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: DOMAIN
<222> LOCATION: (1)...(15)
<223> OTHER INFORMATION: BuNT di-chain loop region

<400> SEQUENCE: 20

Cys Lys Asn Ile Val Ser Val Lys Gly Ile Arg Lys Ser Ile Cys
 1   5   10   15

<210> SEQ ID NO 21
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(5)
<223> OTHER INFORMATION: Bovine enterokinase protease cleavage site.

<400> SEQUENCE: 21

Asp Asp Asp Asp Lys
 1   5

<210> SEQ ID NO 22
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(1)
<223> OTHER INFORMATION: Tobacco Etch Virus protease cleavage site
      consensus sequence
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)...(3)
<223> OTHER INFORMATION: Xaa can be amino amino acid
<221> NAME/KEY: VARIANT
<222> LOCATION: (5)...(5)
<223> OTHER INFORMATION: Xaa can be amino amino acid

<400> SEQUENCE: 22

Glu Xaa Xaa Tyr Xaa Gln Gly

-continued

1 5

<210> SEQ ID NO 23
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(7)
<223> OTHER INFORMATION: Tobacco Etch Virus protease cleavage site consensus sequence
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)...(3)
<223> OTHER INFORMATION: Xaa can be any amino acid
<221> NAME/KEY: VARIANT
<222> LOCATION: (5)...(5)
<223> OTHER INFORMATION: Xaa can be any amino acid

<400> SEQUENCE: 23

Glu Xaa Xaa Tyr Xaa Gln Ser
1 5

<210> SEQ ID NO 24
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(7)
<223> OTHER INFORMATION: Tobacco Etch Virus protease cleavage site.

<400> SEQUENCE: 24

Glu Asn Leu Tyr Phe Gln Gly
1 5

<210> SEQ ID NO 25
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(7)
<223> OTHER INFORMATION: Tobacco Etch Virus protease cleavage site.

<400> SEQUENCE: 25

Glu Asn Leu Tyr Phe Gln Ser
1 5

<210> SEQ ID NO 26
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(7)
<223> OTHER INFORMATION: Tobacco Etch Virus protease cleavage site.

<400> SEQUENCE: 26

Glu Asn Ile Tyr Thr Gln Gly
1 5

<210> SEQ ID NO 27
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(7)
<223> OTHER INFORMATION: Tobacco Etch Virus protease cleavage site.

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<400> SEQUENCE: 27

Glu Asn Ile Tyr Thr Gln Ser
1 5

<210> SEQ ID NO 28
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(7)
<223> OTHER INFORMATION: Tobacco Etch Virus protease cleavage site.

<400> SEQUENCE: 28

Glu Asn Ile Tyr Leu Gln Gly
1 5

<210> SEQ ID NO 29
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(7)
<223> OTHER INFORMATION: Tobacco Etch Virus protease cleavage site.

<400> SEQUENCE: 29

Glu Asn Ile Tyr Leu Gln Ser
1 5

<210> SEQ ID NO 30
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(7)
<223> OTHER INFORMATION: Tobacco Etch Virus protease cleavage site.

<400> SEQUENCE: 30

Glu Asn Val Tyr Phe Gln Gly
1 5

<210> SEQ ID NO 31
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(7)
<223> OTHER INFORMATION: Tobacco Etch Virus protease cleavage site.

<400> SEQUENCE: 31

Glu Asn Val Tyr Ser Gln Ser
1 5

<210> SEQ ID NO 32
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(7)
<223> OTHER INFORMATION: Tobacco Etch Virus protease cleavage site.

<400> SEQUENCE: 32

Glu Asn Val Tyr Ser Gln Gly
1 5

-continued

<210> SEQ ID NO 33
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (0)...(0)
<223> OTHER INFORMATION: Tobacco Etch Virus protease cleavage site.

<400> SEQUENCE: 33

Glu Asn Val Tyr Ser Gln Ser
1 5

<210> SEQ ID NO 34
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(7)
<223> OTHER INFORMATION: human rhinovirus 3C protease cleavage site consensus sequence
<221> NAME/KEY: VARIANT
<222> LOCATION: (1)...(1)
<223> OTHER INFORMATION: Xaa can be amino acid, with D or E preferred
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)...(2)
<223> OTHER INFORMATION: Xaa can be G, A, V, L, I, M, S or T

<400> SEQUENCE: 34

Xaa Xaa Leu Phe Gln Gly Pro
1 5

<210> SEQ ID NO 35
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(7)
<223> OTHER INFORMATION: Human Rhinovirus 3C protease cleavage site.

<400> SEQUENCE: 35

Glu Ala Leu Phe Gln Gly Pro
1 5

<210> SEQ ID NO 36
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(7)
<223> OTHER INFORMATION: Human Rhinovirus 3C protease cleavage site.

<400> SEQUENCE: 36

Glu Val Leu Phe Gln Gly Pro
1 5

<210> SEQ ID NO 37
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(7)
<223> OTHER INFORMATION: Human Rhinovirus 3C protease cleavage site.

<400> SEQUENCE: 37

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Glu Leu Leu Phe Gln Gly Pro
1 5

<210> SEQ ID NO 38
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(7)
<223> OTHER INFORMATION: Human Rhinovirus 3C protease cleavage site.

<400> SEQUENCE: 38

Asp Ala Leu Phe Gln Gly Pro
1 5

<210> SEQ ID NO 39
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(7)
<223> OTHER INFORMATION: Human Rhinovirus 3C protease cleavage site.

<400> SEQUENCE: 39

Asp Val Leu Phe Gln Gly Pro
1 5

<210> SEQ ID NO 40
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (0)...(0)
<223> OTHER INFORMATION: Human Rhinovirus 3C protease cleavage site.

<400> SEQUENCE: 40

Asp Leu Leu Phe Gln Gly Pro
1 5

<210> SEQ ID NO 41
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(6)
<223> OTHER INFORMATION: subtilisin cleavage site consensus sequence
<221> NAME/KEY: VARIANT
<222> LOCATION: (1)...(4)
<223> OTHER INFORMATION: Xaa can be any amino acid

<400> SEQUENCE: 41

Xaa Xaa Xaa Xaa His Tyr
1 5

<210> SEQ ID NO 42
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(6)
<223> OTHER INFORMATION: subtilisin cleavage site consensus sequence
<221> NAME/KEY: VARIANT
<222> LOCATION: (1)...(4)
<223> OTHER INFORMATION: Xaa can be any amino acid

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<400> SEQUENCE: 42

Xaa Xaa Xaa Xaa Tyr His
1 5

<210> SEQ ID NO 43
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(2)
<223> OTHER INFORMATION: subtilisin cleavage site

<400> SEQUENCE: 43

His Tyr
1

<210> SEQ ID NO 44
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(2)
<223> OTHER INFORMATION: subtilisin cleavage site

<400> SEQUENCE: 44

Tyr His
1

<210> SEQ ID NO 45
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(6)
<223> OTHER INFORMATION: subtilisin cleavage site

<400> SEQUENCE: 45

Pro Gly Ala Ala His Tyr
1 5

<210> SEQ ID NO 46
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(6)
<223> OTHER INFORMATION: hydroxylamine cleavage site

<400> SEQUENCE: 46

Asn Gly Asn Gly Asn Gly
1 5

<210> SEQ ID NO 47
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(2)
<223> OTHER INFORMATION: hydroxylamine cleavage site

<400> SEQUENCE: 47

Asn Gly

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1

<210> SEQ ID NO 48
<211> LENGTH: 98
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(98)
<223> OTHER INFORMATION: SUMO/ULP-1 protease cleavage site.

<400> SEQUENCE: 48

Met Ala Asp Ser Glu Val Asn Gln Glu Ala Lys Pro Glu Val Lys Pro
1 5 10 15

Glu Val Lys Pro Glu Thr His Ile Asn Leu Lys Val Ser Asp Gly Ser
20 25 30

Ser Glu Ile Phe Phe Lys Ile Lys Lys Thr Thr Pro Leu Arg Arg Leu
35 40 45

Met Glu Ala Phe Ala Lys Arg Gln Gly Lys Glu Met Asp Ser Leu Arg
50 55 60

Phe Leu Tyr Asp Gly Ile Arg Ile Gln Ala Asp Gln Thr Pro Glu Asp
65 70 75 80

Leu Asp Met Glu Asp Asn Asp Ile Ile Glu Ala His Arg Glu Gln Ile
85 90 95

Gly Gly

<210> SEQ ID NO 49
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: flexible G-spacer

<400> SEQUENCE: 49

Gly Gly Gly Gly
1

<210> SEQ ID NO 50
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: flexible G-spacer

<400> SEQUENCE: 50

Gly Gly Gly Gly Ser
1 5

<210> SEQ ID NO 51
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: flexible A-spacer

<400> SEQUENCE: 51

Ala Ala Ala Ala
1

<210> SEQ ID NO 52
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 52

Tyr Gly Gly Phe Leu
1 5

<210> SEQ ID NO 53
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 53

Tyr Gly Gly Phe Met
1 5

<210> SEQ ID NO 54
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 54

Tyr Gly Gly Phe Met Arg Gly Leu
1 5

<210> SEQ ID NO 55
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 55

Tyr Gly Gly Phe Met Arg Phe
1 5

<210> SEQ ID NO 56
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 56

Tyr Gly Gly Phe Met Arg Arg Val Gly Arg Pro Glu Trp Trp Met Asp
1 5 10 15

Tyr Gln Lys Arg Tyr Gly
20

<210> SEQ ID NO 57
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Necturus maculosus

<400> SEQUENCE: 57

Tyr Gly Gly Phe Met Arg Arg Val Gly Arg Pro Glu Trp Trp Leu Asp
1 5 10 15

Tyr Gln Lys Arg Tyr Gly
20

<210> SEQ ID NO 58
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Bombina orientalis

<400> SEQUENCE: 58

Tyr Gly Gly Phe Met Arg Arg Val Gly Arg Pro Glu Trp Trp Gln Asp
1 5 10 15

Tyr Gln Lys Arg Tyr Gly
20

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<210> SEQ ID NO 59
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Xenopus laevis

<400> SEQUENCE: 59

Tyr Gly Gly Phe Met Arg Arg Val Gly Arg Pro Glu Trp Trp Glu Asp
1 5 10 15

Tyr Gln Lys Arg Tyr Gly
20

<210> SEQ ID NO 60
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Neoceratodus forsteri

<400> SEQUENCE: 60

Tyr Gly Gly Phe Met Arg Arg Val Gly Arg Pro Glu Trp Lys Leu Asp
1 5 10 15

Asn Gln Lys Arg Tyr Gly
20

<210> SEQ ID NO 61
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Danio rerio

<400> SEQUENCE: 61

Tyr Gly Gly Phe Met Arg Arg Val Gly Arg Pro Asp Trp Trp Gln Glu
1 5 10 15

Ser Lys Arg Tyr Gly
20

<210> SEQ ID NO 62
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 62

Tyr Pro Trp Phe
1

<210> SEQ ID NO 63
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 63

Tyr Pro Phe Phe
1

<210> SEQ ID NO 64
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 64

Tyr Gly Gly Phe Met Thr Ser Glu Lys Ser Gln Thr Pro Leu Val Thr
1 5 10 15

<210> SEQ ID NO 65
<211> LENGTH: 10

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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 65

Tyr Gly Gly Phe Leu Arg Lys Tyr Pro Lys
 1 5 10

<210> SEQ ID NO 66
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 66

Tyr Gly Gly Phe Met Thr Ser Glu Lys Ser Gln Thr Pro Leu Val Thr
 1 5 10 15

Leu Phe Lys Asn Ala Ile Ile Lys Asn Ala Tyr Lys Lys Gly Glu
            20 25 30

<210> SEQ ID NO 67
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 67

Tyr Gly Gly Phe Met Ser Ser Glu Lys Ser Gln Thr Pro Leu Val Thr
 1 5 10 15

Leu Phe Lys Asn Ala Ile Ile Lys Asn Ala His Lys Lys Gly Gln
            20 25 30

<210> SEQ ID NO 68
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 68

Tyr Gly Gly Phe Leu Arg Lys Tyr Pro
 1 5

<210> SEQ ID NO 69
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 69

Tyr Gly Gly Phe Met Thr Ser Glu Lys Ser Gln Thr Pro Leu Val Thr
 1 5 10 15

Leu

<210> SEQ ID NO 70
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 70

Tyr Gly Gly Phe Leu Arg Arg Ile Arg Pro Lys Leu Lys Trp Asp Asn
 1 5 10 15

Gln

<210> SEQ ID NO 71
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 71

Tyr Gly Gly Phe Leu Arg Arg Ile Arg Pro Lys Leu Lys
1 5 10

<210> SEQ ID NO 72
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 72

Gly Gly Phe Leu Arg Arg Ile Arg Pro Lys Leu Lys Trp Asp Asn Gln
1 5 10 15

<210> SEQ ID NO 73
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 73

Gly Gly Phe Leu Arg Arg Ile Arg Pro Lys Leu Lys
1 5 10

<210> SEQ ID NO 74
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Xenopus laevis

<400> SEQUENCE: 74

Tyr Gly Gly Phe Leu Arg Arg Ile Arg Pro Lys Leu Arg Trp Asp Asn
1 5 10 15

Gln

<210> SEQ ID NO 75
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Xenopus laevis

<400> SEQUENCE: 75

Tyr Gly Gly Phe Leu Arg Arg Ile Arg Pro Arg Leu Arg Trp Asp Asn
1 5 10 15

Gln

<210> SEQ ID NO 76
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Protopterus annectens

<400> SEQUENCE: 76

Tyr Gly Gly Phe Met Arg Arg Ile Arg Pro Lys Ile Arg Trp Asp Asn
1 5 10 15

Gln

<210> SEQ ID NO 77
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Danio rerio

<400> SEQUENCE: 77

Tyr Gly Gly Phe Met Arg Arg Ile Arg Pro Lys Leu Arg Trp Asp Asn
1 5 10 15

Gln

-continued

<210> SEQ ID NO 78
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Anguilla rostrata

<400> SEQUENCE: 78

Tyr Gly Gly Phe Met Arg Arg Ile Arg Pro Lys Leu Lys Trp Asp Ser
 1   5   10   15

Gln

<210> SEQ ID NO 79
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 79

Tyr Gly Gly Phe Leu Arg Arg Gln Phe Lys Val Val Thr Arg Ser Gln
 1   5   10   15

Glu Asp Pro Asn Ala Tyr Ser Gly Glu Leu Phe Asp Ala
            20   25

<210> SEQ ID NO 80
<211> LENGTH: 28
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 80

Tyr Gly Gly Phe Leu Arg Arg Gln Phe Lys Val Val Thr Arg Ser Gln
 1   5   10   15

Glu Asn Pro Asn Thr Tyr Ser Glu Asp Leu Asp Val
            20   25

<210> SEQ ID NO 81
<211> LENGTH: 28
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 81

Tyr Gly Gly Phe Leu Arg Arg Gln Phe Lys Val Val Thr Arg Ser Gln
 1   5   10   15

Glu Ser Pro Asn Thr Tyr Ser Glu Asp Leu Asp Val
            20   25

<210> SEQ ID NO 82
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Cavia porcellus

<400> SEQUENCE: 82

Tyr Gly Gly Phe Leu Arg Arg Gln Phe Lys Val Val Thr Arg Ser Gln
 1   5   10   15

Glu Asp Pro Asn Ala Tyr Ser Glu Glu Phe Phe Asp Val
            20   25

<210> SEQ ID NO 83
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Sus scrofa

<400> SEQUENCE: 83

Tyr Gly Gly Phe Leu Arg Arg Gln Phe Lys Val Val Thr Arg Ser Gln
 1   5   10   15

-continued

Glu Asp Pro Asn Ala Tyr Tyr Glu Glu Leu Phe Asp Val
20 25

<210> SEQ ID NO 84
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Canis familiaris

<400> SEQUENCE: 84

Tyr Gly Gly Phe Leu Arg Arg Gln Phe Lys Val Val Thr Arg Ser Gln
1 5 10 15

Glu Asp Pro Asn Ala Tyr Ser Gly Glu Leu Leu Asp Gly
20 25

<210> SEQ ID NO 85
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Bos taurus

<400> SEQUENCE: 85

Tyr Gly Gly Phe Leu Arg Arg Gln Phe Lys Val Val Thr Arg Ser Gln
1 5 10 15

Glu Asp Pro Ser Ala Tyr Tyr Glu Glu Leu Phe Asp Val
20 25

<210> SEQ ID NO 86
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Bufo marinus

<400> SEQUENCE: 86

Tyr Gly Gly Phe Leu Arg Arg Gln Phe Lys Val Thr Thr Arg Ser Glu
1 5 10 15

Glu Asp Pro Ser Thr Phe Ser Gly Glu Leu Ser Asn Leu
20 25

<210> SEQ ID NO 87
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Bombina orientalis

<400> SEQUENCE: 87

Tyr Gly Gly Phe Leu Arg Arg Gln Phe Lys Val Thr Thr Arg Ser Glu
1 5 10 15

Glu Glu Pro Gly Ser Phe Ser Gly Glu Ile Ser Asn Leu
20 25

<210> SEQ ID NO 88
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Xenopus laevis

<400> SEQUENCE: 88

Tyr Gly Gly Phe Leu Arg Arg Gln Phe Lys Val Asn Ala Arg Ser Glu
1 5 10 15

Glu Asp Pro Thr Met Phe Ser Asp Glu Leu Ser Tyr Leu
20 25

<210> SEQ ID NO 89
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Xenopus laevis

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<400> SEQUENCE: 89

Tyr Gly Gly Phe Leu Arg Arg Gln Phe Lys Val Asn Ala Arg Ser Glu
1 5 10 15

Glu Asp Pro Thr Met Phe Ser Gly Glu Leu Ser Tyr Leu
20 25

<210> SEQ ID NO 90
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Polypterus senegalus

<400> SEQUENCE: 90

Tyr Gly Gly Phe Leu Arg Arg His Phe Lys Ile Ser Val Arg Ser Asp
1 5 10 15

Glu Glu Pro Ser Ser Tyr Ser Asp Glu Val Leu Glu Leu
20 25

<210> SEQ ID NO 91
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Danio rerio

<400> SEQUENCE: 91

Tyr Gly Gly Phe Leu Arg Arg His Phe Lys Ile Ser Val Arg Ser Asp
1 5 10 15

Glu Glu Pro Ser Ser Tyr Glu Asp Tyr Ala Leu
20 25

<210> SEQ ID NO 92
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Anguilla rostrata

<400> SEQUENCE: 92

Tyr Gly Gly Phe Leu Arg Arg His Phe Lys Ile Ser Val Arg Ser Asp
1 5 10 15

Glu Glu Pro Gly Ser Tyr Asp Val Ile Gly Leu
20 25

<210> SEQ ID NO 93
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Neoceratodus forsteri

<400> SEQUENCE: 93

Tyr Gly Gly Phe Leu Arg Arg His Phe Lys Ile Thr Val Arg Ser Asp
1 5 10 15

Glu Asp Pro Ser Pro Tyr Leu Asp Glu Phe Ser Asp Leu
20 25

<210> SEQ ID NO 94
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Oncorhynchus masou

<400> SEQUENCE: 94

Tyr Gly Gly Phe Leu Arg Arg His Tyr Lys Leu Ser Val Arg Ser Asp
1 5 10 15

Glu Glu Pro Ser Ser Tyr Asp Asp Phe Gly Leu
20 25

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<210> SEQ ID NO 95
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 95

Tyr Gly Gly Phe Leu Arg Arg Gln Phe Lys Val Val Thr
1 5 10

<210> SEQ ID NO 96
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Bufo marinus

<400> SEQUENCE: 96

Tyr Gly Gly Phe Leu Arg Arg Gln Phe Lys Val Thr Thr
1 5 10

<210> SEQ ID NO 97
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Xenopus laevis

<400> SEQUENCE: 97

Tyr Gly Gly Phe Leu Arg Arg Gln Phe Lys Val Asn Ala
1 5 10

<210> SEQ ID NO 98
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Polypterus senegalus

<400> SEQUENCE: 98

Tyr Gly Gly Phe Leu Arg Arg His Phe Lys Ile Ser Val
1 5 10

<210> SEQ ID NO 99
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Neoceratodus forsteri

<400> SEQUENCE: 99

Tyr Gly Gly Phe Leu Arg Arg His Phe Lys Ile Thr Val
1 5 10

<210> SEQ ID NO 100
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Oncorhynchus masou

<400> SEQUENCE: 100

Tyr Gly Gly Phe Leu Arg Arg His Tyr Lys Leu Ser Val
1 5 10

<210> SEQ ID NO 101
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 101

Phe Gly Gly Phe Thr Gly Ala Arg Lys Ser Ala Arg Lys Arg Lys Asn
1 5 10 15

Gln

-continued

<210> SEQ ID NO 102
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 102

Phe Gly Gly Phe Thr Gly Ala Arg Lys Ser Ala Arg Lys Leu Ala Asn
1 5 10 15

Gln

<210> SEQ ID NO 103
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 103

Phe Gly Gly Phe Thr Gly Ala Arg Lys Ser Ala Arg Lys Tyr Ala Asn
1 5 10 15

Gln

<210> SEQ ID NO 104
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 104

Phe Gly Gly Phe Thr Gly Ala Arg Lys Ser Ala
1 5 10

<210> SEQ ID NO 105
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 105

Phe Gly Gly Phe Thr Gly Ala Arg Lys Tyr Ala
1 5 10

<210> SEQ ID NO 106
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 106

Phe Gly Gly Phe Thr Gly Ala Arg Lys Ser Tyr
1 5 10

<210> SEQ ID NO 107
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 107

Phe Gly Gly Phe Thr Gly Ala Arg Lys Ser Ala Arg Lys
1 5 10

<210> SEQ ID NO 108
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 108

Met Pro Arg Val Arg Ser Leu Phe Gln Glu Gln Glu Glu Pro Glu Pro

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1 5 10 15

Gly Met Glu Glu Ala Gly Glu Met Glu Gln Lys Gln Leu Gln
20 25 30

<210> SEQ ID NO 109
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 109

Phe Ser Glu Phe Met Arg Gln Tyr Leu Val Leu Ser Met Gln Ser Ser
1 5 10 15

Gln

<210> SEQ ID NO 110
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 110

Thr Leu His Gln Asn Gly Asn Val
1 5

<210> SEQ ID NO 111
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: flexible A-spacer

<400> SEQUENCE: 111

Ala Ala Ala Ala Val
1 5

<210> SEQ ID NO 112
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(5)
<223> OTHER INFORMATION: Consensus sequence for a SUMO/ULP-1 protease
cleavage site
<221> NAME/KEY: VARIANT
<222> LOCATION: (3)...(5)
<223> OTHER INFORMATION: Xaa can be any amino acid

<400> SEQUENCE: 112

Gly Gly Xaa Xaa Xaa
1 5

<210> SEQ ID NO 113
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(7)
<223> OTHER INFORMATION: Consensus sequence for a Tobacco Vein Mottling
Virus protease cleavage site
<221> NAME/KEY: VARIANT
<222> LOCATION: (1)...(2)
<223> OTHER INFORMATION: Xaa can be any amino acid

<400> SEQUENCE: 113

Xaa Xaa Val Arg Phe Gln Gly
1 5

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<210> SEQ ID NO 114
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(7)
<223> OTHER INFORMATION: Consensus sequence for a Tobacco Vein Mottling Virus protease cleavage site
<221> NAME/KEY: VARIANT
<222> LOCATION: (1)...(2)
<223> OTHER INFORMATION: Xaa can be any amino acid

<400> SEQUENCE: 114

Xaa Xaa Val Arg Phe Gln Ser
1 5

<210> SEQ ID NO 115
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(7)
<223> OTHER INFORMATION: Tobacco Vein Mottling Virus protease cleavage site

<400> SEQUENCE: 115

Glu Thr Val Arg Phe Gln Gly
1 5

<210> SEQ ID NO 116
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (0)...(0)
<223> OTHER INFORMATION: Tobacco Vein Mottling Virus protease cleavage site

<400> SEQUENCE: 116

Glu Thr Val Arg Phe Gln Ser
1 5

<210> SEQ ID NO 117
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(7)
<223> OTHER INFORMATION: Tobacco Vein Mottling Virus protease cleavage site

<400> SEQUENCE: 117

Asn Asn Val Arg Phe Gln Gly
1 5

<210> SEQ ID NO 118
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(7)
<223> OTHER INFORMATION: Tobacco Vein Mottling Virus protease cleavage site

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<400> SEQUENCE: 118

Asn Asn Val Arg Phe Gln Ser
1 5

<210> SEQ ID NO 119
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(5)
<223> OTHER INFORMATION: Consensus sequence for a non-human Caspase 3 protease cleavage site
<221> NAME/KEY: VARIANT
<222> LOCATION: (2)...(2)
<223> OTHER INFORMATION: Xaa can be any amino acid with E preferred
<221> NAME/KEY: VARIANT
<222> LOCATION: (3)...(3)
<223> OTHER INFORMATION: Xaa can be any amino acid
<221> NAME/KEY: VARIANT
<222> LOCATION: (5)...(5)
<223> OTHER INFORMATION: Xaa can be any amino acid with G or S preferred

<400> SEQUENCE: 119

Asp Xaa Xaa Asp Xaa
1 5

<210> SEQ ID NO 120
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(5)
<223> OTHER INFORMATION: Non-human Caspase 3 protease cleavage site

<400> SEQUENCE: 120

Asp Glu Val Asp Gly
1 5

<210> SEQ ID NO 121
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(5)
<223> OTHER INFORMATION: Non-human Caspase 3 protease cleavage site

<400> SEQUENCE: 121

Asp Glu Val Asp Ser
1 5

<210> SEQ ID NO 122
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(5)
<223> OTHER INFORMATION: Non-human Caspase 3 protease cleavage site

<400> SEQUENCE: 122

Asp Glu Pro Asp Gly
1 5

<210> SEQ ID NO 123
<211> LENGTH: 5
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<400> SEQUENCE: 123

Asp Glu Pro Asp Ser
1 5

<210> SEQ ID NO 124
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<400> SEQUENCE: 124

Asp Glu Leu Asp Gly
1 5

<210> SEQ ID NO 125
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Asp Glu Leu Asp Ser
1 5

What is claimed:

1. A method of treating urogenital-neurological disorder in a human, the method comprising the step of administering to the human in need thereof a therapeutically effective amount of a composition including a modified Clostridial toxin comprising an opioid peptide binding domain, a Clostridial toxin translocation domain and a Clostridial toxin enzymatic domain,

wherein the urogenital-neurological disorder is selected from the group consisting of urinary incontinence, overactive bladder, detrusor dysfunction, lower urinary tract dysfunction, urinary retention and urinary hesitancy,

wherein administration of the composition reduces a symptom of the urogenital-neurological disorder, thereby treating the human.

2. The method of claim **1**, wherein the modified Clostridial toxin comprises a linear amino-to-carboxyl single polypeptide order of 1) the Clostridial toxin enzymatic domain, the Clostridial toxin translocation domain, the opioid peptide binding domain, 2) the Clostridial toxin enzymatic domain, the opioid peptide binding domain, the Clostridial toxin translocation domain, 3) the opioid peptide binding domain, the Clostridial toxin translocation domain, and the Clostridial toxin enzymatic domain, 4) the opioid peptide binding domain, the Clostridial toxin enzymatic domain, the Clostridial toxin translocation domain, 5) the Clostridial toxin translocation domain, the Clostridial toxin enzymatic domain and the opioid peptide binding domain, or 6) the Clostridial toxin translocation domain, the opioid peptide binding domain and the Clostridial toxin enzymatic domain.

3. The method of claim **1**, wherein the opioid peptide binding domain is an enkephalin, a BAM22 peptide, an endomorphin, an endorphin, a dynorphin, a nociceptin or a hemorphin.

4. The method of claim **1**, wherein the Clostridial toxin translocation domain is a BoNT/A translocation domain, a BoNT/B translocation domain, a BoNT/C1 translocation domain, a BoNT/D translocation domain, a BoNT/E translocation domain, a BoNT/F translocation domain, a BoNT/G translocation domain, a TeNT translocation domain, a BaNT translocation domain, or a BuNT translocation domain.

5. The method of claim **1**, wherein the Clostridial toxin enzymatic domain is a BoNT/A enzymatic domain, a BoNT/B enzymatic domain, a BoNT/C1 enzymatic domain, a BoNT/D enzymatic domain, a BoNT/E enzymatic domain, a BoNT/F enzymatic domain, a BoNT/G enzymatic domain, a TeNT enzymatic domain, a BaNT enzymatic domain, or a BuNT enzymatic domain.

6. A method of treating urogenital-neurological disorder in a human, the method comprising the step of administering to the human in need thereof a therapeutically effective amount of a composition including a modified Clostridial protein comprising an opioid peptide binding domain, a Clostridial toxin translocation domain, a Clostridial toxin enzymatic domain, and an exogenous protease cleavage site,

wherein the urogenital-neurological disorder is selected from the group consisting of urinary incontinence, overactive bladder, detrusor dysfunction, lower urinary tract dysfunction, urinary retention and urinary hesitancy, and

wherein administration of the composition reduces a symptom of the urogenital-neurological disorder, thereby treating the human.

7. The method of claim **6**, wherein the modified Clostridial toxin comprises a linear amino-to-carboxyl single polypeptide order of 1) the Clostridial toxin enzymatic domain, the exogenous protease cleavage site, the Clostridial toxin translocation domain, the opioid peptide binding domain, 2) the Clostridial toxin enzymatic domain, the exogenous protease cleavage site, the opioid peptide binding domain, the Clostridial toxin translocation domain, 3) the opioid peptide binding domain, the Clostridial toxin translocation domain, the exogenous protease cleavage site and the Clostridial toxin enzymatic domain, 4) the opioid peptide binding domain, the Clostridial toxin enzymatic domain, the exogenous protease cleavage site, the Clostridial toxin translocation domain, 5) the Clostridial toxin translocation domain, the exogenous protease cleavage site, the Clostridial toxin enzymatic domain and the opioid peptide binding domain, or 6) the Clostridial toxin translocation domain, the exogenous protease cleavage site, the opioid peptide binding domain and the Clostridial toxin enzymatic domain.

8. The method of claim **6**, wherein the opioid peptide binding domain is an enkephalin, a BAM22 peptide, an endomorphin, an endorphin, a dynorphin, a nociceptin or a hemorphin.

9. The method of claim **6**, wherein the Clostridial toxin translocation domain is a BoNT/A translocation domain, a BoNT/B translocation domain, a BoNT/C1 translocation domain, a BoNT/D translocation domain, a BoNT/E translocation domain, a BoNT/F translocation domain, a BoNT/G translocation domain, a TeNT translocation domain, a BaNT translocation domain, or a BuNT translocation domain.

10. The method of claim **6**, wherein the Clostridial toxin enzymatic domain is a BoNT/A enzymatic domain, a BoNT/B enzymatic domain, a BoNT/C1 enzymatic domain, a BoNT/D enzymatic domain, a BoNT/E enzymatic domain, a BoNT/F enzymatic domain, a BoNT/G enzymatic domain, a TeNT enzymatic domain, a BaNT enzymatic domain, or a BuNT enzymatic domain.

11. The method of claim **6**, wherein the exogenous protease cleavage site is a plant papain cleavage site, an insect papain cleavage site, a crustacian papain cleavage site, an enterokinase cleavage site, a human rhinovirus 3C protease cleavage site, a human enterovirus 3C protease cleavage site, a tobacco etch virus protease cleavage site, a Tobacco Vein Mottling Virus cleavage site, a subtilisin cleavage site, a hydroxylamine cleavage site, or a Caspase 3 cleavage site.

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